



Interactions between fungal plant pathogens on leaves. Especially simultaneous development of *Rhynchosporium secalis* and *Drechslera teres* on barley

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Risø-PhD-20(EN)

Interactions Between Fungal Plant Pathogens on Leaves.

Especially simultaneous development of *Rhynchosporium secalis* and *Drechslera teres* on barley



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November 2005

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Title: Interactions between fungal plant pathogens on leaves.
Especially simultaneous development of *Rhynchosporium secalis*
and *Drechslera teres* on barley

Department: Biosystems Department

This thesis is submitted in partial fulfilment of the requirements for the Ph.D. degree at the Royal Veterinary and Agricultural University (KVL), Copenhagen, Denmark.

Abstract:

Plant diseases caused by fungi are major potential threats to yield in both organic and conventional cereal production, and generally several species of pathogenic fungi are found together on the same plants in the field. This PhD thesis concludes, that interaction between different foliar fungal species may influence total disease levels and often via negative effects. This implies that less disease is observed where several diseases occur together, relative to where they occur individually. Such interaction effects have often been ignored in the past, as plant diseases are traditionally studied as independent events. The thesis provides an important review of available literature, considering the theoretical background for analyzing foliar disease interactions as well as previously published data studies. Difficulties in distinguishing different types of competition are emphasized. The thesis considers the barley diseases scald (*Rhynchosporium secalis*) and net blotch (*Drechslera teres*). Aiming to understand how diseases interact in the field, it is concluded that increased focus should be placed on considering the dynamics of plant growth along with epidemiological development. This is pointed out by observations of antagonism between scald and net blotch on individual leaves and via a simulation model. The model shows, that difference in pathogen dispersal rate between leaf layers of the plant is important for competitive outcome from two species. Detailed disease observations on individual leaves does not, though, give a better description of yield loss from disease, relative to plot assessment, where disease severities are determined as averages over the crop. The work presented in the PhD thesis is relevant to all who work with foliar pathogens. Optimal disease control and plant breeding is based on knowledge of factors determining disease development and hence interactions between simultaneously developing pathogens must be considered.

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Scald and net blotch in a barley crop.
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Preface

This thesis is submitted as partial fulfilment of the requirements for the degree of Philosophiae Doctor (PhD) at the Royal Veterinary and Agricultural University (KVL), Copenhagen, Denmark.

The PhD project has been carried out between the Department of Plant Biology, KVL; Biosystems Department, Risø; and Research Centre Flakkebjerg, Danish Institute of Agricultural Sciences (DIAS). Employment has been at Risø where the majority of work has taken place and the field trial was carried out in Flakkebjerg.

The PhD project was funded by the project Characteristics of Spring Barley Varieties for Organic Farming¹ (BAR-OF) under the Danish Research Centre for Organic Farming II (DARCOF).

A PhD is supposed to be a learning process and considered as such, the previous three years have been a major success: I have acquired a considerable amount of knowledge and new skills. Before I started this project, I had never paid much attention to the origin of brownish wilted spots on plant leaves and 'organic' to me, was the 'Ø'² on foodstuff in the supermarket that implied the product had not been stuffed with unnecessary chemicals and the animals had lived a 'good life'.

Some of the activities, which have helped fill my considerable knowledge gaps, are participation in courses on plant pathology (KVL) and plant disease epidemics (NOVA) and presentation of my work at the 11th International Cereal Rust and Powdery Mildew Conference in Norwich, UK, 2004. Furthermore, I have participated in meetings of the BAR-OF project, where my work has been presented and discussed and I have participated in two European meetings in the Cost action: SUSVAR³. These activities have allowed me to meet a great range of people and be presented to other peoples research.

Another important part of the three years of study has been the membership of the Research School for Organic Agriculture and Food Systems⁴ (SOAR), where I participated in two week-long summer-schools and six bi-annual seminars (each 2 days long). The activities in SOAR are aimed at PhD-student working within organic farming from various disciplines. The activities have revolved around organic farming in general, including discussions of research methodologies in relation to principles and practice of organic agriculture and relations between sustainability and organic farming.

Through the data processing, I have spent quite some time on learning new software s and developing my data handling and analytical skills.

From the above follows the imminent question: how much of this is reflected in the present thesis? The thesis presents the outcome of the actual scientific project, and is a reflection of three years of work with, data analysis of and not least discussions regarding interaction between fungal plant pathogens on leaves. The papers focus on accumulating existing knowledge on this subject, analysis of actual data and theoretical problems associated with the exploration of interactions between foliar pathogens, along

1 <http://www.darcof.dk/research/darcofii/vi2.html>

2 The Ø is the Danish state label for certified organic products

3 <http://cost860.dk/>

4 <http://www.soar.dk>

with consideration of disease assessment in the field. The organic scenery behind the project is not strongly reflected in the thesis. The focus has been on the basic approaches of studying simultaneously developing diseases in the field, more than the agricultural practises and the associated plant pathological problems. In the near future though, the thesis will be summarised to a contribution for DARCOFenews and FØJØenyt, electronic newsletters on Danish research in organic farming, in English and Danish respectively, communicating this to a wider audience.

The thesis is structured as follows:

Chapter 1

Introduction including objectives

Chapter 2

Vollmer JH; Østergård H; Pinnschmidt HO; Munk L (in prep). Interaction based on density dependence versus other interaction types between foliar fungal pathogens. Prepared for submission to **European Journal of Plant Pathology**

Chapter 3

Vollmer JH; Østergård H; Pinnschmidt HO; Munk L (submitted). Simultaneous epidemic development of scald (*Rhynchosporium secalis*) and net blotch (*Drechslera teres*) on individual leaf layers of a spring barley crop. Submitted to **Plant Pathology**

Chapter 4

Vollmer JH; Østergård H (in prep). Effect of leaf layer dynamics and vertical spore dispersal on competition between foliar pathogens. Prepared for submission to **Ecological Modelling** or **Phytopathology**

Chapter 5

Pinnschmidt HO; Vollmer JH; Munk L. Methods for assessing net blotch severity on spring barley and their relations to grain weight. Extended abstract.

Chapter 6

Outlook

Appendix:

An abstract and two posters prepared during the PhD, which are not to be considered in the evaluation of the thesis.

Tables and figures appear at the end of each chapter

My name is on the front cover of this thesis, and I am responsible for the content. However, I could not have carried out this work had it not been for a great number of people in my surroundings, of which some deserve special acknowledgements.

First and foremost I would like to acknowledge my supervisors, of which I have had no less than three: Hanne Østergård (Risø), Hans Pinnschmidt (DIAS) and Lisa Munk (KVL). They have all engaged and shown a genuine interest in the welfare of the project, and for this I am grateful. Through numerous meetings over a very considerable amount of cake and associated hot beverages, we have had long and fruitful discussions, which have among others served to illustrate how differently, different people may approach the same problem. Other than this, they have each contributed in different ways to the project. Hans was a much appreciated and patient tutor during the field trial and my first acquaintance with fungal pathogens 'live', he has provided thorough critique on the different stages of manuscripts, and his analytical expertise has been vital for the last paper of the thesis. Lisa has assured that all formalities with KVL were followed, provided a valuable link to the university and given important feed back on the general process including the structure of the thesis. Hanne was my 'everyday supervisor' and through the years, we have had ongoing long discussions that have served to set the fundament of the thesis. Regardless of her packed schedule, she has insisted that we discuss the progression of the project and always taken the time to answer questions. She has been a vital support through her continued patience, encouragement and enthusiasm.

While carrying out the field trial in Flakkebjerg, Lis E. Henriksen provided important technical assistance as a general introduction to the work with fungi and in the preparation of field inoculum. Ib M. Skovgaard, Department of Natural Sciences, KVL, provided valuable suggestions on data analysis. For this I am grateful to both of them.

Finally, but no less important: I have had three good years at Risø, and would like to express a general gratitude to all those who made the time outside the office, for general fun, teas & coffees, lunch and supportive friendship.

Jeanette Hyldal Vollmer

Roskilde, November 2005

Summary of PhD thesis

It is common to find several pathogenic species together on crop plants in the field and several studies have shown that interspecific interactions can effect the total disease level. Optimal disease control and plant breeding is based on knowledge of factors determining epidemic development and hence the importance of simultaneously developing pathogens must be considered. A pathogen grows on single leaves of the canopy; and the crop thereby represents a dynamic substrate with leaves appearing and being removed over the season. This is therefore the level of the crop which disease must be assessed at to gain maximum information on disease dynamics.

The thesis focus is on interaction between foliar pathogenic fungi in the field by considering the theoretical background for analysing the data on simultaneously developing pathogens, reviewing published interaction studies and considering the influence of canopy structure for pathogen competition and disease assessment. The term interaction is used as a general description for any type of relation between two pathogen species. The interaction type where two pathogens promote development of each other is referred to as synergism. Negative interactions between two species is generally referred to as competition, which can be either antagonistic (interference competition) or density dependent (exploitation competition)

Literature on interactions between foliar fungal pathogens is considered and discussed in a review paper. Special emphasis is placed on formulating the theoretical background to explore these interactions. From the reviewed studies, it is concluded that while interactions may have synergistic effects, it is competition that is mainly observed, and it is often difficult to decide whether this arises from antagonism or density dependence. Both the trophic interaction of the pathogen (bio- or necro-) with the host as well as timing of the respective pathogen arrival is important. It is emphasized, though, that more research on interactions is needed from field trials with naturally developing diseases.

Simultaneous development of the barley diseases scald (caused by *Rhynchosporium secalis*) and net blotch (caused by *Drechslera teres*) were studied in a field trial, where plants were collected over the season, and disease severity assessed on preserved single leaves. Diseases were found to have negative associations on single leaves. Further, maximum disease levels of either disease were often lower on individual leaf layers when both pathogens had been inoculated. These effects were not so strong though, as to be evidenced as non-additive effects of the two pathogens on the total disease level. This study thus showed antagonistic interaction between the two pathogens species at field level. The single leaf approach provided information not evident at field level, which may give an important insight into dynamics of disease and crop development.

A simulation model for the simultaneous development of two pathogens was used to explore the importance of canopy dynamics (by describing leaf area as discrete leaf layers over time) and pathogen epidemic parameters (including dispersal) on the competitive outcome by only considering exploitation competition. By showing that the competitive outcome from two simultaneously developing pathogen species is sensitive to differences in dispersal rates within the canopy and the overlap of leaf layers over time, the model illustrated that different competitive outcomes may be reached based on non-antagonistic effects. Difficulties associated with drawing conclusions regarding

antagonistic effects between pathogens based only disease development data on plot level are emphasized.

Disease assessment is often performed as a severity measure of the amount of green leaf area covered by disease. One problem with this method is that it ignores the relationship between the dynamics of crop and disease development. In the field trial where leaves had been preserved, disease was also recorded at crop level, and this offered the opportunity to compare results obtained by the two methods. Thousand grain weight was used as a yield estimate and this was correlated to disease estimates obtained by the two methods at various growth stages (GS). The results indicate that disease assessments at GS 70 are appropriate to reflect whole-season severity levels of net blotch and that the time consuming single-tiller method is in this respect not superior to the simpler whole-plot method. However, assessing individual leaf layers allowed observation of the epidemic development in great detail. This showed, for example, how much each leaf layer contributed at any given time to the total disease level and revealed that a substantial fraction of the total disease is being removed during the course of an epidemic by senescence of the lower leaves. This level of detail in examining the dynamics of epidemics cannot be achieved by the whole-plot method.

The work presented in this thesis is relevant for all who work with foliar pathogens. The thesis provides an important outline of the theoretical background to analysing foliar disease interactions, through which the difficulties in distinguishing antagonism and density dependence are emphasized. The thesis underlines the importance of studying species interactions when working with disease, and of considering the relation between crop and disease dynamics. More specific research is needed on multiple disease development, both under controlled conditions as well as, and most importantly, in the field.

Sammendrag af PhD afhandling

Det er almindeligt at der i en afgrøde forekommer flere sygdomme samtidigt, og en række studier har vist, at vekselvirkninger imellem disse kan have indflydelse på det samlede sygdomsniveau. Idet optimal sygdomsforebyggelse og –bekæmpelse er baseret på viden om de faktorer, der har indflydelse på epidemisk udvikling, bør betydningen af disse typer vekselvirkninger, være kendte. Bladpatogener i en afgrøde vokser på enkelte blade der vokser frem og dør igen i løbet af vækstsæsonen. Dette har betydning for patogenets udvikling, og bladet er derfor det rette niveau at studere sygdomme og deres vekselvirkninger på.

I denne afhandling sættes fokus på vekselvirkninger mellem bladpatogene svampe i marken ved at belyse såvel den teoretiske baggrund for analyse af samtidigt forekommende patogener samt den eksisterende litteratur af emnet og betydningen af afgrødens dynamik for konkurrence imellem patogenerne og sygdomsbedømmelse i marken. Vekselvirkning (*interaction*) anvendes som en general term til at beskrive alle forhold imellem to patogenarter. Vekselvirkningen betegnes synergisme hvor de to arter fremmer hinandens udvikling. Konkurrence dækker alle typer af gensidige negative vekselvirkninger, og disse kan inddeles i to grupper afhængigt af mekanismen bag konkurrencen: antagonisme (*interference competition*) og tæthedsafhængighed (*exploitation competition*).

Publicerede studier af vekselvirkninger mellem patogene svampe på blade diskuteres i en oversigtsartikel. Der er her lagt særlig vægt på at formulere den teoretiske baggrund for analyse af data på vekselvirkninger mellem patogene svampe på blade. Både positive og negative vekselvirkninger er observerede, dog med klar overvægt af de sidste, og i mange af disse studier har man ikke kunnet adskille hvorvidt de negative vekselvirkninger er opstået ved antagonisme eller tæthedsafhængighed. Både svampens trofiske interaktion med værtsplanten (bio-eller necrotrof) såvel som den relative tilførsel til værtsplanten, viser sig at være vigtigt for forholdet imellem de to svampe. Fra denne oversigt understreges det, at yderligere forskning i vekselvirkninger mellem patogene svampe er nødvendig, og at denne bør tage udgangspunkt i naturligt forekommende sygdomme i marken.

Samtidig udvikling af de to bygsygdomme skoldplet (forårsaget af *Rhynchosporium secalis*) og bladplet (forårsaget af *Drechslera teres*) blev studeret i et markforsøg, under hvilket planter blev indsamlet henover en sæson, og angrebsniveauet blev bedømt på individuelle tørrede og pressede blade. Resultaterne viste en negativ sammenhæng mellem forekomsten af de to sygdomme på enkelte blade. Derudover blev det observeret, at det maksimale sygdomsniveau på de enkelte bladetaager ofte var lavere, når begge patogener var blevet påført. Disse effekter var dog ikke så stærke, at de kunne ses som ikke-additive effekter af de to patogener på det samlede sygdomsniveau. Dette forsøg har således vist en antagonistisk vekselvirkning i mellem de to svampe, og bedømmelse af sygdom på individuelle blade har vist sig at give informationer, som ikke kan observeres på afgrødeniveau og kan derfor hjælpe til at give yderligere indsigt i forholdet mellem afgrødens udvikling og sygdomsdynamikken.

En simuleringsmodel beskrev den samtidige udvikling af to bladpatogener og blev brugt til at stille spørgsmål omkring betydningen af afgrødedynamik (ved at beskrive bladareal som diskrete bladetaager over tid) og patogenets epidemiske parametre (inkl. vertikal

spredning.) for konkurrenceforholdet i mellem de to arter, ved kun at lade tæthedsafhængighed indgå som vekselvirkning. Det blev vist, at konkurrenceforholdet i mellem samtidigt udviklende bladpatogene svampe var følsomt for forskelle i spredningsrater og livslængden af bladetager, hvoraf følger, at der i marken kan forventes forskellige konkurrencemæssige resultater, baseret på disse faktorer alene. Dette understreger problemerne ved at drage konklusioner angående ikke-tæthedsafhængige vekselvirkninger fra markdata.

Et ofte anvendt mål for bedømmelse af sygdom er det procentvise sygdomsdække relativt til hele afgrøden. Et problem med denne metode er, at den ignorerer dynamikken i sygdomsudvikling i forhold til afgrødens udvikling. I markforsøget hvor enkeltblade blev indsamlet til sygdomsbedømmelse, blev der samtidigt bedømt sygdom per parcel, hvilket gav mulighed for at sammenligne resultaterne fra de to metoder. Tusindkornsvægt (TKV) blev brugt som mål for udbyttet, og blev korreleret til en række forskellige sygdomsmål ved forskellige vækststadier (GS) af planten. Resultaterne herfra indikerer, at sygdomsbedømmelse taget ved GS 70 er passende afspejlinger af sygdomsniveau over hele sæsonen for bladplet, og den forklarer TKV lige så godt som enkeltbladsmetoden. Det skal dog understreges, at bedømmelsen på enkelte blade muliggjorde et mere detaljeret studie af den epidemiske udvikling. Dette viste bl.a. hvor meget de enkelte etager på forskellige tidspunkter bidrog til den samlede mængde sygdom, og heraf kunne det konstateres, at en væsentlig del af den samlede mængde sygdom i løbet af en sæson fjernes når de nederste blade visner. Viden om dynamikken af en epidemi på dette niveau kan ikke opnås ved den traditionelle afgrødebedømmelse.

Artiklerne i denne afhandling er relevante for alle, som arbejder med bladpatogener. Afhandlingen giver en vigtig præsentation af den teoretiske baggrund for analyse af vekselvirkning mellem patogener på blade, hvorigennem problemer i forhold til at adskille tæthedsafhængige og antagonistiske interaktioner understreges. Afhandlingen understreger vigtigheden af at tage hensyn til vekselvirkninger i forbindelse med sygdomsobservationer og epidemiudviklinger. Det understreges, at mere forskning omkring udvikling af sygdomme og deres vekselvirkninger, både under kontrollerede forhold, og vigtigst, under naturlige forhold i marken, er nødvendig.

1 Introduction

1.1 Plants, Pathogens & People

The relation between fungi and plants evolved at an early stage in the evolution towards the world we see today. Fungi and plants probably entered land together some 700 million years ago (Heckman *et al.*, 2001) and a diverse array of relationships have since formed between the two kingdoms, both mutualistic and parasitic. The relationship between people and plants started to develop to its present form, some 10.000 years ago when the domestication of plants was initiated (Ladizinsky, 1998), a relation which is strongly influenced by the plant-fungal bond.

In nature, the dynamic relations between plants and their pathogens are under control by a co-evolutionary relationship, a process whereby the host and the pathogen species contribute reciprocally to the forces of natural selection they exert on each other (Begon *et al.*, 1996). This natural dynamic relationship is interfered with in agricultural production in two ways: the dynamics over time of the host species are not determined by natural selective mechanisms, but rather by the choices of the farmer; and the spatial distribution is dramatically altered from any natural distributions, such that when the host is available, it is in a form which offers ample opportunity for pathogen proliferation. This implies that when insisting on growing plants as is done under any agricultural practice, aiming to maximise yield, plant pathological problems are inevitable. The challenge lies in operating under these circumstances.

Research towards maximising plant yield in relation to diseases is focused at two sides, improving plant properties like resistance, which are not considered in this thesis, and understanding the disease epidemiology. Improving the understanding of pathogen epidemiology is aimed at devising management strategies to help avoid the pathogens in space and time. This approach is based on the assumption, that success of disease control is increased when based on knowledge of underlying processes rather than depending on methods evolved through experience (Berger, 1977).

1.2 Organic farming & plant pathology

The host-pathogen relationship between plant and fungi is a fundamental biological relationship, but how this relationship is allowed to progress in different situations depends on agricultural practices. Within plant production a variety of forms are found; at one end of the scale are third-world low input systems where natural phenomena play crucial roles in determining the outcome, and at the other end are high-input agricultural systems where, crudely speaking, the only biology relied upon is the ability of the seed to develop into a plant. Somewhere in between these extremes, organic farming may be placed. In the western part of the world, this agricultural practise enjoys the same commodities (machinery, research etc) as the conventional types, but is founded on a wider environmental perspective. Part of the definition for organic farming in the Nordic countries is : "Organic farming describes a self-sustaining and persistent agro-ecosystem in good balance. As far as possible, the system is based on local and renewable resources. It builds on a holistic view that incorporates the ecological, economical and social aspects of agricultural production in both the local and global perspectives. " (DARCOF, 2000). According to the DARCOF homepage⁵ this implies a distinction from

⁵ <http://www.darcof.dk>

conventional agriculture "by exercising particular respect for the environment, nature, and animal welfare, etc."

From a plant production perspective, the organic farming definition implies, among others, that chemical inputs are generally not allowed, that nutrient levels are different from conventional agriculture and pests cannot be removed, which the agricultural practise must be designed in accord with. The effect is that management aiming to avoid diseases relies on an understanding of the natural processes influencing disease development and relies on the design of preventive measures to avoid pests.

The holistic emphasis has implications for science in organic agriculture, where the system considerations in the agricultural practice also requires a system orientated research approach. Insisting on holism and systems approaches begs the question, what is the system? In the perspective of organic agriculture, this is at least the individual management unit, i.e. a farm or perhaps a collection of such, between which resources are shared. For foliar pathogens the most important aspects are probably related to keeping the inoculum potential down by discarding infected seeds thereby reducing transmission via seeds, good crop rotations by growing the same host plant in different fields in different years, and the use of suitable crop varieties with optimal resistance properties, and even better the use of variety mixtures to increase the genetic diversity facing the pathogen in the crop. All of these management tools can only be optimised if disease biology is understood.

In a biological perspective the system may be described as the environment with influence on the species, the size of which depends on the organisms studied such that it may differ considerably between soil pathogens and foliar pathogens with high rates of wind dispersal. In many cases there is not one correct level of system definition, but rather this depends on the questions explored. No two farms, years or pathogens are the same, and considering variation which arises from the combination of these, it is clear that what is needed, is to understand general principles to operate in such complex systems, rather than aiming to device specific solutions. An aspect of what is important to understand is how levels of a given disease may influence levels of others.

1.3 Multiple diseases

Early research on multiple diseases and their interactions was provided by Fawcett (1931), who observed that "Nature does not work with pure cultures alone but most frequently with associations". He proceeded to discuss studies of multiple diseases from various plant-pathogen systems, which showed that interaction was evident between pathogens and encouraged the continued research on this subject. This approach was further promoted by Kranz *et.al.* (1989), who termed it a synecological approach, where the complete pest-situation (weeds, pathogens, insects ect.) is considered when working with plant pathology.

This was also addressed by Johnson (1990) who considered the analysis of multiple pest species. He emphasized the importance of developing a greater understanding on how pests interact to affect yield. A part of this is looking at specific interactions, accumulating knowledge to form theoretical basis for analysis as well-defined expectations are lacking.

Attention has thus been on the importance of pest interactions for at least three quarters of a decade, but a common basis with respect to knowledge and analytical guidelines is still absent. A major reason for this, is perhaps related to the remark made by Fawcett

(1931) "work with mixtures [of pathogens], however, will not make the already complex problem of plant pathology as a whole any easier or less complex... ". The subject seems not to have been reviewed previously, other than briefly summarised by Johnson (1990), who made a preliminary survey of studies dealing with interactions between pests and damage on yield responses, and found that the majority of studies concluded an antagonistic interaction between the species.

Exploration of this subject may be approached at different levels of interspecific organisation. A high level approach uses statistical analyses of complete cropping systems, incorporating data on all disease causing organisms, inputs and other management related factors as well as climatic variables to understand factors affecting yield, like those by Savary and colleagues who have applied this type of analysis to characterise injury profiles in a region in India (Savary *et al.*, 1997) and across Asia (Savary *et al.*, 2000).

At the other end of the scale is the consideration of disease complexes, a term used here to describe disease from closely associated pathogens, e.g. maize tarspot (Hock *et al.*, 1992) or Fusarium ear blight, a disease caused by several species between which synergistic interactions may occur (Xu *et al.*, 2005).

In between these, is consideration of processes affecting pathogens in the same field at the same time, these may not be dependent on the presence of each other, but given that the same host species is exploited they are likely to be affected by presence of one another. It is interactions at this level of organisation, which is the focus in the present thesis, the exploitation of the same leaf resource by different species of fungal pathogens.

The objective in studies of co-developing diseases is often to establish whether synergistic or antagonist effects operate to influence disease levels from several simultaneously developing pathogen species. Distinguishing these types is a non-trivial analytical challenge, as even in the absence of antagonistic interactions, effects from the two are expected to be non-additive, because as pointed out by Padwick (1956; from Johnson, 1990): "one pest cannot affect what another has already damaged". This was also recognised by Kranz *et.al.* (1989) who saw the difficulties in distinguishing predisposition and direct competition in the field. This emphasizes that the analysis or theoretical background must clearly formulated prior to analysis of disease development data.

1.4 Presentation of Pathogens

The two fungi *Drechslera teres* and *Rhynchosporium secalis*, pathogens of barley, are considered as model pathogens in three of the papers in this thesis and they are therefore presented by a general description below. Environmental factors play a crucial role for growth of fungi. Temperature and humidity along with resistance properties and nutritional status of the host jointly play a major role in determining epidemic potential of the primary inoculum and disease development in a field. This is reflected in the summary below, where observations by different authors even under well-controlled environmental conditions are affected by pathotypes and host varieties used. This introduction serves to give a general introduction to these fungi through a presentation of their biologies with focus on the part of their life cycles that cause the disease epidemics.

1.4.1 *Drechslera teres*

Net blotch is caused by the ascomyte *Pyrenophora teres* Drechs., conidial form *Drechslera teres* (Sacc.) Shoemaker. *Pyrenophora teres* has been divided into two forms by Smedegård (1971) based on differences in symptoms on barley leaves: the net type *P. teres* f. *teres* and the spot-type *P. teres* f. *maculata* Smedeg. Only the net type is considered here.

The main host is barley (*Hordeum vulgare*), but the pathogen may also be found on other species of *Hordeum*, oat (*Avena* sp.), wheat (*Triticum* sp.) and Brome (*Bromus*) and can infect a range of other grasses. Wild *Hordeum* is the only other host though, which seems to have practical importance in net blotch epidemiology (Shipton *et al.*, 1973; Brown *et al.*, 1993).



Net blotch

The fungus persists between seasons as seedborne mycelium or pseudothecia in plant debris from where conidia or ascospores are produced, or it may be introduced via infected seeds (Steffenson, 1997). The primary inoculum serves to infect plants, from where lesions develop and conidia are produced which serve as secondary inoculum. Ascocarp development is slow, and this stage mainly serves a role in the maintenance of genetic diversity and thus development of new virulences, more so than in the disease progression (Shipton *et al.*, 1973). The disease development within a field over a season is largely caused by the anamorph, *D. teres*, conidia producing part of the fungal life cycle, and this is therefore the focus below.

The extremes of the temperature range under which conidia germination has been seen, are 2°C (Shaw, 1986) and 33°C (Steffenson, 1997), with optimum somewhere in the range 15-25°C (Singh, 1963; Shaw, 1986; Van den Berg & Rossnagel, 1990; Steffenson, 1997). Germination requires the presence of water or 100% relative humidity but has no relation to light (Shaw, 1986).

A 90 % germination rate within 3 hours was observed by Shaw (1986) of which 29 % of applied spores resulted in infection and successful lesion growth, though on young leaves 80% of germinated conidia may form infections (Shaw, 1986).

Germ tubes from conidia usually arise from the central cells and give rise to appressoria-like structures. The penetrating hypha passes through the epidermal cell and enlarges slightly on passing through the lower cell wall. Development of hyphae is then intercellular with cell death occurring in advance of the fungus. Conidia are generally formed from the necrotic leaf areas. Conidiophores usually arise direct from between epidermal cells or, more rarely, from stomata and occur singly or in groups of not more than two or three. The lesions of net blotch on susceptible hosts are characterised by a dark brown reticulate pattern developing in an otherwise light brown lesion. The growth rate of lesions was observed by Shaw (1986) who observed the highest rate at 1.6 mm day⁻¹

The duration from infection to sporulation, the latent period, may range from 8-25 days decided by temperature, but generally within the range 10-14 days in the temperature range 15-25°C (Shaw, 1986). Higher temperatures and relative humidity decrease the latent period. Spore release is found to have positive correlation with temperature and negative with humidity and leaf wetness (Martin *et al.*, 1984).

The variability in temperature optima for epidemic parameters, has lead to the suggestion, that there may be ecotypes among the pathogenic fungi, which will explain the difference between pathotypes found on cereals in North America on summer-grown crops versus *e.g.* the Mediterranean region grown over mild winters (Shipton *et al.*, 1973).

1.4.2 *Rhynchosporium secalis*

Scald or barley leaf blotch is caused by the haploid imperfect fungi (Deuteromycete) *Rhynchosporium secalis* (Oudem.) J. J. Davis, *i.e.* no teleomorph has been described for the fungus. However, the species has a very high genetic diversity (Mcdermott *et al.*, 1989), which does not correspond well with purely asexual development and from genetic analyses it seems that some genetic recombination occurs (Salamati *et al.*, 2000) and the fungus is likely to be heterothallic (Linde *et al.*, 2003; Foster & Fitt, 2004),

R. secalis is not a very specialized pathogen, and can use a range of hosts. Barley (*Hordeum vulgare*) is a major host species, but the pathogen may also be found on a large range of related genera of grasses (*Poaceae*), *e.g.* *Agropyron*, *Bromus*, *Elymus*, *Lolium*, and *Secale* (Beer, 1991).



Photo by H.O. Pinnschmidt

Scald

In the absence of a sexual life stage the fungal life cycle is comprised by conidia production, host infection and hyphal growth. Micronidia produced in flask-shaped branches of older parts of the mycelium have been observed by Skoropad & Grinchenko (1957). Attempts to germinate these however failed (Skoropad & Grinchenko, 1957) and no function has been reported. The recent findings, which indicate that the fungus is heterothallic (Linde *et al.*, 2003; Foster & Fitt, 2004), imply that sexual reproduction does take place in this species but no sexual fruiting bodies have been described.

In the absence of any known sexual structures, the fungi probably survives between cropping seasons as mycelia in infected host residues, but may also be transmitted via seeds (Jackson, 1997). However, left over residues from previous years crops are considered the most important source of primary inoculum (Nielsen & Jensen, 2001). Sporulating potential of fungal material on crop residues left in the field could survive for up to 12 months (Murray *et al.*, 1999). Overwintering mycelia will produce spores when environmental conditions are favourable, serving as primary inoculum to initiate an epidemic.

Conidia are two-celled and characteristically beak-shaped and germination optimum is reported between 15-21°C and at least 95 % air humidity (Beer, 1991). Germination of up to 80 % of spores occurs within 24 hours (Ryan & Clare, 1975) when conditions are dark and moist (Jackson, 1997). The conidia can germinate with several germ tubes from one or both cells and appresoria develop at the tips of the germ tubes (Ayesu-Offei, 1970; Shipton *et al.*, 1974). Penetration happens directly by penetrating the cuticle (Ayesu-Offei, 1970). No data have been found for infection success of *R. secalis*. Infection is followed by formation of a subcuticular mycelium, which develops into a stroma, one to several cells in thickness. Later, hyphae penetrate the epidermal cell layer, particularly at the junction of guard and epidermal cells. Infection causes stomata to open more to light, due to an alteration of the turgor relations between guard cells and the surrounding epidermal cells. Infection causes mesophyll cells to collapse, which is evident on the leaf surface as water soaking and scalding of the tissues. Formation of

conidia on the stroma results in the separation and eventual cracking of the cuticle, thus superficially exposing the stroma.

The latent period has been reported between 8 and 14 days at 20°C and about twice as long at 5°C (Beer, 1991; Jackson, 1997). Lesion growth rate has been observed at 2 mm day⁻¹ (Xue & Hall, 1991). Conidia production is reported to be poor beyond 5 and 30°C and retarded between 27 and 37°C with optimum between 15 and 20°C (Jackson, 1997)

Ayesu-Offei (1971) counted conidia production, and recorded $0.5-1.3 \times 10^6$ conidia produced in 48 hours, from groups of 2-3 lesions collected in the field and allowed to sporulate in the laboratory under optimal conditions. Skoropad (1966) found that a scald lesions ceased to sporulate after 18 days at 18°C at alternating wet and dry periods.

Spore release is favoured by rainfall and wind following rainfall, confirming that the conidia are splash dispersed and with rain may be picked up by the wind (Ayesu-Offei & Carter, 1971). The same authors did not trap any significant amount of spores in traps over the fields, implying that long distance dispersal is of minor importance. Distance of splash dispersed *R. secalis* conidia in a rain tower was measured by Fitt *et al.* (1988) who found that from leaves ca 25 % of the spores reached 10 cm and very few went beyond 20 cm above the leaf they were produced on.

1.5 Thesis Objectives

The general topic of this thesis is interactions between foliar fungal pathogens on leaves and how co-occurring species affect the disease levels of each other. It is apparent from above, that the subject would benefit from the generation of a summarized knowledgebase, which can serve as basis for adequate formulation of hypotheses and help set directions of future research.

The objectives of the present thesis are formulated as follows:

- Review available information on interaction between foliar fungal pathogens on leaves to generate an overview of available studies from which general patterns of interaction types between species may be found.
- Consider the theoretical basis for analysis of data on interaction between foliar pathogens, with focus on how to establish occurrence of different interaction types. This includes hypotheses as well as consideration of how to monitor diseases relative to crop development.
- A more specific objective from the above, is to establish whether interaction occurs between the two barley pathogens *Rhynchosporium secalis* and *Drechslera teres* in the field.

These objectives are pursued via accumulation of literature on the subject, a field study on the two specific pathogens, a simulation model exploring development of two foliar pathogens in the absence of any synergistic or antagonistic effects to form the background expectations for interaction considerations. Last, two general disease assessment methodologies are compared to gain further information on the relation between crop development and observed disease levels.

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2 Interaction between fungal plant pathogens on leaves

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2.1 Abstract

Multiple diseases are expected to become an increasingly important issue when both demand to reduce pesticide input and organic areas are increasing. Interactions between foliar fungal plant pathogens have been the focus of a number of studies, but a synthesis of the results is missing.

This review concerns foliar pathogens on their host species and covers all kinds of plant-pathogen systems. The review concludes that pathogen interactions can affect total disease level in the field, and that antagonistic as well as synergistic effects may be seen. The time of arrival of a pathogen is an important factor in the response of a multiple pathogen system along with fungus nutritional association with the host. A large proportion of the studies explore pathogens under controlled environmental conditions with a constant climate and no other stresses on the plant. Such studies tend to show more pronounced effects than field trials. Therefore, the necessity of further field studies of interactions between naturally developing pathogens is emphasized.

An outline of the theoretical background to analysing interactions between foliar pathogens and their resulting disease levels is presented. For foliar pathogens, dependent on leaf area, it is a non-trivial task to distinguish interference competition (antagonism) and exploitation competition (density dependence). This distinction is rarely made in the published studies and subsequently often confused.

2.2 Keywords

Density dependence, multiple diseases, exploitation competition, interference competition

2.3 Introduction

The development of a plant is subjected to a variety of challenges from its environment, which should all be considered when aiming to grow the plants to maximise a yield. An important part of the environment is biotic stresses, generally considered as pests. These come from a range of biological groups, including other plants (weeds), insects and

microorganisms such as fungi. Many pests have been studied as independent phenomena, but over the years there is increasing consensus that these must be considered together, as interactions between components of the pests may have an important influence on the reaction of the plant (Fawcett, 1931; 1989; Savary *et al.*, 2000). A sound theoretical basis is lacking with respect to knowledge of these interaction and means of analysis (Fawcett, 1931; Kranz & Jörg , 1989; Johnson, 1990) This is a challenge that needs to be approached from several sides.

An important component of pest complexes in the field is fungal pathogens. The aim of this paper is to present and discuss the available literature on interaction between foliar fungal pathogens and discuss approaches to analyse the results of these. The literature on this subject is considerable, but yet the studies appear independently ranging over at least 70 years, however, with few researchers having published more than a single paper. To be able to ask increasingly qualified questions within the area it is fundamental that this information is collected and discussed.

The term interaction is a general term to describe all types of relations between two simultaneously occurring species. A set of generic terms have been defined to describe the range of different positive or negative interspecific relations (Table 2:1), *e.g.* trivial indifference (0/0), mutualism (+/+), and competition (-/-) (Christiansen & Fenchel , 1977). Different disciplines often have different words for the same phenomena, and in plant pathology the situation where two pathogens promote disease or yield reduction caused by each other, is referred to as synergism (Table 2:1). The term competition describes a situation where two species are negatively affected by each other, but dependent on how this occurs, the term is divided into two different kinds: Interference (direct inhibition, when both species actively inhibit each other) and exploitation (indirect inhibition, when a common resource is in short supply) (Odum, 1971). For foliar diseases these two terms are important to distinguish. Interference competition describes the situation where physiological processes drive the interaction, *e.g.* by direct chemical interaction or induced resistance, in plant pathology also referred to as antagonism. Exploitation competition describes the situation where the pathogen growth rate is reduced with increasing density of either species, simply due to a reduced number of suitable infection sites, also known as density dependence, which describes the influence of decreasing resource availability with increasing density of the resource-consumer on growth of the population of this (Begon *et al.*, 1996)(Table 2:1). The important difference is, that for interference competition intra-and interspecific effects are different and in exploitation competition these are similar.

For two co-developing pathogens it is expected they will affect each other, at least by exploitation competition given an overlap in their used resource, but the objective is more often to explore whether interference competition takes place, either via direct chemical interaction or induced resistance. Distinguishing these types is a non-trivial analytical challenge, as even in the absence of antagonistic interactions, effects from the two are expected to be non-additive, because as pointed out by Padwick (1956; from Johnson, 1990): "one pest cannot affect what another has already damaged". This was also recognised by Kranz (1989) who saw the difficulties in distinguishing predisposition and direct competition in the field.

The focus here is foliar fungal pathogens as these have some common characteristics, which are important for data analysis and conclusions regarding interactions. They infect the leaf, develop within this to various degrees and disperse spores from its surface. Disease levels are most often estimated as disease severity, the proportion of the surface

area covered with disease. This dependence on the leaf area resource makes them comparable across patho-systems.

The review only includes studies of pathogens developing on susceptible host plants. To be included in the review, a study must be based on experimental data in a set-up that considers development of pathogen species or pathotypes, individually as well as together. The above choices exclude studies of development in proportions of pathogens, either species or pathotypes, over time as effects from interaction cannot be distinguished from *e.g.* differences in climatic optima. Also not included, are studies dealing with biological control and induced resistance by non-pathogenic micro-organisms or induced susceptibility by otherwise non-pathogenic pathotypes. The paper generally takes a pathogen perspective, effects on the plant receive little attention and direct yield loss studies are not included. Studies were intended included from all plant-pathogen systems, where foliar pathogens are present simultaneously, but in reality all originate from crop-plants, a research bias probably driven by economic incentives.

The paper includes an outline of the theoretical background to analysis of foliar disease data, which presents some of the challenges associated with exploring interaction and particularly to make the distinction between interference and exploitation competition from disease data. This is followed by an outline of the results from studies of pathogen interactions. Finally, important factors and general principles are discussed.

2.3.1 Note on nomenclature

The classification of the fungi is an ongoing task and several of the pathogens studied have been reclassified and thus reported under different names. In the present paper the current name is used, irrespective of use in original literature. Changes from the cited literature are given Table 2:2. Only the disease causing stage of the pathogen, generally the anamorph, is referred to, *e.g.* where the pathogen is presented as *Cochliobolus sativus* in the literature this is referred to as *Bipolaris sorokiniana* in the review. The terms pathotype and forma specialis (f. sp.) are used to cover all taxonomic units below the species level, which may else be referred to by strain or race.

Another development is in the description of plant growth stage (GS) development. Feekes' scale (Feekes, 1941; Large, 1954) was used earlier but has later been replaced by the growth stages described by Zadoks *et al.* (1974). Here, the stage described by the original authors is given along with the GS according to the translation between the two scales by Zadoks *et al.* (1974)

2.4 Theoretical background for analysis of interactions

This section outlines the theoretical background for analysis of interaction between foliar diseases. First is given a presentation of problems associated with common experimental designs, mainly related to inoculation densities. This is followed by considerations of disease growth particular to foliar diseases and consequent problems associated with formulation of hypotheses to distinguish types of interaction.

2.4.1 Inoculation

Exploring interaction between two species often involves a design where the two pathogens are observed alone versus together. This type of design can be either additive or by replacement (Figure 2:1). In additive designs competition is tested by comparing results obtained from species *A* and *B* alone versus the same two together in the same original densities, *i.e.* intraspecific densities are the same in all treatments, but the total density is the sum of the two single densities when they are observed together. In a

replacement design the density of the individual species in the two-species treatment are 50% of the treatments with only the single species, such that the total density is constant

A more elaborate version of the replacement design is the de Wit series, originating in plant studies (de Wit, 1960). Yields of each species are compared under a range of relative input densities and presented relative to yield in the single species situation (Figure 2:2). The yield observations of *A* and *B* at the various input proportions are presented relative to their yield when observed alone (RY_A and RY_B). The relative yield total (RYT) is the sum of the two at the range of input proportions. The analysis compares the observed values to the straight null lines, which correspond to a situation with equal inter- and intraspecific competition, according to the model. Observed lines below the null-lines thus indicate a stronger inter- than intraspecific competition and observed lines above the null lines indicate that intraspecific competition is stronger than inter.

The two types of design have been discussed by Snaydon (1991), who points out the importance of density on species performance. For a trait of a species, *e.g.* spore production, the intraspecific inoculation density will influence the amount of spores produced per original inoculated spore (Damgaard & Østergård, 1996), with a non-linear response curve, which can have different shapes, *e.g.* asymptotic or quadratic (Figure 2:3). Therefore, for observations made at the two different intraspecific densities, *e.g.* D1 and D2 (Figure 2:3) different numbers of spores are produced. The consequence is that in a replacement design, where the two species are studied at the same total density but different intraspecific densities, the effects from differences in the latter may be confounded with interspecific competition. Additive designs do not have this problem, and Snaydon (1991) suggest that the ultimate design to investigate interspecific interaction by, is a bivariate factorial design where the two potential competitors are studied at a range of intraspecific and total density levels.

The method of de Wit replacement series has been explored for species with complex life cycles by Newton *et al.* (1998). Simulations of a model with distinct life cycle stages under three density levels, illustrated that differences in life history parameters (*e.g.* infection efficiency or maximum number of lesions that a competitor can produce on a leaf) influence the relative production lines (RY_A and RY_B). The authors did conclude though, that observations of significant deviations from the null RYT lines are good indications of differences in intra- and interspecific competition, while the opposite is not the case.

2.4.2 Growth

The importance of the leaf surface for disease development means that density dependence or exploitation competition always must be considered when analysing disease interactions on leaf surfaces. The theoretical absolute maximum proportion of a leaf that can express disease symptoms is 1 (or 100% severity), which has implications for hypotheses regarding simultaneously developing pathogens and analysis of disease data.

The logistic model is used as a simple model to describe levels of two diseases (*A* and *B*; Eq. 1) developing on separate plants, *i.e.* no interaction is possible. K_A and K_B are the maximum levels (=1) of either disease, which have different rates of growth ($r_A=0.15$ and $r_B=0.20$).

$$\begin{aligned}
\frac{dA}{dt} &= r_A \times A \left(1 - \frac{A}{K_A} \right) \\
\frac{dB}{dt} &= r_B \times B \left(1 - \frac{B}{K_B} \right)
\end{aligned}
\tag{1}$$

The graphical presentation from these, over a time span of 100 days based on an initial amounts (A_0 , B_0) both equal to 0.01, is shown in Figure 2:4a. The figure shows how A and B both reach their maximum level, but at different points in time based on their rate of growth.

Simulating the same two species developing over the same leaf area, the two equations are linked to accommodate the inherent competition for leaf area between the two species. The maximum total disease severities of both are still 1, but growth of each species is now influenced by the sum of the two disease levels.

$$\begin{aligned}
\frac{dA}{dt} &= r_A \times A \left(1 - \frac{A+B}{K_A} \right) \\
\frac{dB}{dt} &= r_B \times B \left(1 - \frac{A+B}{K_B} \right)
\end{aligned}
\tag{2}$$

The result using the same parameter values is shown in Figure 2:4b where total disease level reaches 1 and non of the two diseases reach their potential maximum, but lower asymptotic levels determined by their growth rates. This simple model illustrates, that making the observation that levels of A and B observed alone (Figure 2:4a) versus together (Figure 2:4b) are different, does not in itself indicate that any interference competition or antagonism occurs, but can simply be ascribed to density dependence. In situations where levels of developed disease are compared, there is thus no simple null-hypothesis to test antagonism against, *i.e.* expected disease levels in the two-species situation can not be described as a simple function of the single species observations.

An extension of the two coupled logistic equations (Eq. 2) is the Lotka-Volterra two-species competition model (Volterra, 1926; Lotka, 1932). This model includes a competition coefficient (α_{ij}) that determines the weight of influence of i on j , and thereby describes interference competition, and not only exploitation competition.

$$\begin{aligned}
\frac{dA}{dt} &= r_A \times A \left(1 - \frac{A + \alpha_{BA}B}{K_A} \right) \\
\frac{dB}{dt} &= r_B \times B \left(1 - \frac{\alpha_{AB}A + B}{K_B} \right)
\end{aligned}
\tag{3}$$

A value of $\alpha_{ij} = 0$ implies no interaction, or independent developments, and the Lotka-Volterra model is reduced to two independent logistic equations (Eq. 3). For $\alpha_{ij} = 1$ the influence of i on j is described by the same weight it has in itself and density dependence is the only operating effect (Eq. 2). For $\alpha_{ij} < 1$ the effect of a unit of i on j is less than a unit of j and vice versa. The outcome of competition is given by relation between values of K and α , shown theoretically in general ecology textbooks (Begon *et al.*, 1996). This shows that there are areas of values for these parameters where one species will control the other and some, where co-existence is possible (Table 2:3).

The foliar pathogen-host system has a number of characteristics, which mean that some parameter spaces of the Lotka-Volterra model must be employed with caution. The simplest general assumptions behind a model of interaction between two foliar pathogen species are: 1) The competed resource is leaf area and all leaf area is equally suitable for infection by both pathogens; 2) area infected cannot become de-infected and the two diseases cannot overgrow each other. The effect of these is that the amount of either disease cannot decrease, *i.e.* once used, area cannot become available for either pathogen again and a value for α_{ij} of zero is therefore not possible for pathogens developing on the same leaf area. Furthermore, in any simulation using Lotka-Volterra type models, the simulation must include a condition that does not allow a negative change, *i.e.* $dA/dt < 0$ or $dB/dt < 0$.

For any test of interaction the null-hypothesis must be $\alpha_{ij} = 1$, meaning density dependence will affect amount of total final disease. Fitting the Lotka-Volterra equations to data on two simultaneously developing diseases and obtaining α_{ij} -values > 0 does not in itself imply antagonistic effects, or inhibition competition, but may merely reflect expected density dependent effects.

The Lotka-Volterra model with α_{ij} values over 1 simulates antagonism, or interference competition. This situation is shown in Figure 2:4c, where for both competitors α is 2, and the consequent total level of disease is lower than 1, *i.e.* due to the antagonistic effects even the total disease levels do not reach the total possible level.

2.4.3 Additivity

From the above follows, that when data on total disease from two simultaneously developing pathogens are compared to data from their independent developments, additive levels from the single-species situations are not expected in the two-species situation. Rather, exploitation competition is expected to influence total disease levels. A simple example: observing severity of A at 65% and B of 55% from single species developments does not lead to an expectation of 120% severity in the two-species situation.

2.5 Experimental Studies

Studies that consider pathogen development cover both one generation and development over several generations. The former are represented by studies that consider arrival of the pathogen on the host and the immediate developments are monitored, *e.g.* by diseased area or spores produced. The latter consider pathogen interaction effects on epidemic development, aiming to describe disease development over consecutive generations. Studies have been carried out under controlled environmental conditions as well as in the field.

In this section, studies are grouped under three headings, according to the trophic host relation of the studied pair of pathogens, *i.e.* biotrophic-biotrophic, biotrophic-necrotrophic and necrotrophic-necrotrophic.

2.5.1 Biotrophic-Biotrophic

The two major groups of biotrophic pathogens are the fungi causing the rust and mildew diseases, although only *B. graminis* f.sp. *hordei* and *P. hordei* on barley have been studied in combination under the objective of describing their interaction. They have mainly been studied under controlled environmental conditions (Simkin & Wheeler, 1974; Round & Wheeler, 1978; Kiessling & Hoffman, 1985b; Olesen, 2005), while

observations on development in the field have been made only by Simkin & Wheeler (1974).

The greenhouse studies by Simkin & Wheeler (1974), Round & Wheeler (1978), and Kiessling & Hoffmann (1985b) have established that generally pre-inoculation with one of these pathogens reduces the development of the other. This effect is increasing with increasing duration (0-6 days) between the inoculations and with increasing inoculum dose. For *P. hordei* this resulted in fewer and smaller pustules (Round & Wheeler, 1978). The interaction may not be only negative though, as Round and Wheeler (1978) found more pustules from the *P. hordei* inoculation relative to control leaves where *B. graminis* f.sp. *hordei* had been inoculated within the preceding 24 hours. The two pathogens have been studied microscopically by Olesen (2005) where *B. graminis* f.sp. *hordei* was applied 72 hours after *P. hordei* on the first developed leaf of barley plants. Infection success at cell level was monitored and they found that infection success of *B. graminis* was negatively affected by the prior infection of *P. hordei* in cells adjacent to the cells where *B. graminis* f.sp. *hordei* attempted infection. This finding thus confirmed that the formerly applied species interrupts development of the latter. They further found that this effect could be ascribed to intercellular contact in the plant such that the effect is present without physical contact between hyphae of the two fungal species.

Simkin *et al.* (1974) observed developments of *B. graminis* f.sp. *hordei* and *P. hordei* over the growth season on single leaves in the field. Diseases developed from natural inoculum and development was controlled using specific fungicides such that either or both pathogens were allowed to develop. *B. graminis* f.sp. *hordei* developed earlier than *P. hordei* and to much higher levels (up to 40% and below 5 % severity respectively) on the upper 4 leaves. Rates of growth were determined between observation dates and these suggested that *P. hordei* had a negative effect on rates of growth.

Generally, from the studies on *B. graminis* f.sp. *hordei* and *P. hordei* it seems clear that either of the two have potential to effect development of the other. Results have shown though, that even when the first pathogen is inoculated only a few hours before the second, a significant effect in terms of reduced numbers of pustules from the second pathogen is found. This combined with the microscopic observations by Olesen (2005) shows that the interference interaction is evident and the mechanism is mediated via the plant.

2.5.2 Biotrophic-Necrotrophic

Interactions between biotrophic and necrotrophic fungi have been the subject of a larger group of studies. This is a special combination, where one pathogen is dependent on living cells for survival, while the other has devastating effect on host tissue.

Pathogen interactions on bean (*Phaseolus vulgaris*) have been the focus of a two studies. In a greenhouse experiment Lopes & Berger (2001) applied *Uromyces appendiculatus* and *Colletotrichum lindemuthianum* either alone or in various relative concentrations of either of the two pathogens, to obtain dataset with a range of disease levels. Diseases were assessed relative to the leaf area and gas exchange measurements were taken from the bean leaves. These two measures were compared using a model describing the relation between disease severity and photosynthetic rate. For a single pathogen species (A), the photosynthetic rate in the absence of disease (P_0) is related to the rate at disease level x_A (P_{xA}) and β_A presents the ratio between the visually lesioned area and the actual effect on the plant, the virtual lesion size (Bastiaans, 1991).

$$P_{x_A} = P_0 \times (1 - x_A)^{\beta_A} \quad (4)$$

The equation has been extended to accommodate situations where disease is caused by two species, *A* and *B*. This model is based on an assumption of random distribution between the two pathogens over leaf surfaces, and hence ignores the influence of exploitation competition between the two species at higher severity levels.

$$P_{x_{AB}} = P_0 \times (1 - x_A)^{\beta_A} \times (1 - x_B)^{\beta_B} \quad (5)$$

The analysis compares the β values obtained in the two situations, and interaction is concluded based on differences in these. Lopes & Berger (2001) found no significant differences in the obtained β values for either disease between the single and two-pathogen situations, from which they concluded that no interaction was evident.

Interaction between *Phaeoisariopsis griseola* and *U. appendiculatus* on common bean has been studied via its effect on leaf area index duration (de Jesus *et al.*, 2001a) and gas exchange (de Jesus *et al.*, 2001b). The papers presumably reflect the same experiment, based in the field and diseases were initiated from artificial inoculum, either as single species or together. Disease severity was recorded along with various plant physiological properties. The data were analysed by a model which tested additive effects of the pathogens. The conclusion was that the results seemed to indicate an antagonistic effect of the diseases. Recorded severity values were maximum 8% for *P. griseola* and less than 1.5 % for *U. appendiculatus*. These values are so low, that independent disease distributions may be assumed, which could indicate an interference type interaction between the two.

On wheat, Weber *et al.* (1994) observed development of *B. graminis* and *S. nodorum* in a greenhouse experiment and two field trials in different years, all based on artificial inoculum and disease observations performed over ca. 50 days. In the greenhouse experiment *B. graminis* was applied at GS 23 and *S. tritici* at GS 30 and disease development was observed on single marked tillers. Significantly higher levels of *B. graminis* developed in the absence of *S. nodorum* (57 vs. 8% severity), where as levels of *S. nodorum* were not significantly different in the presence and absence of *B. graminis* (average 59 %), based on the inoculated four leaf layers, as *S. nodorum* never dispersed to upper leaves.

In the field *B. graminis* was introduced via infected plants placed in the plots at GS 32 and a *S. nodorum* spore suspension was sprayed over the plots at GS 37. Conditions were unfavourable to *B. graminis*, but the effect of *S. nodorum* found in the greenhouse was evident even at these low levels, with severities of single leaf layers ranging between 0.6 % (least favourable year, in the presence of *S. nodorum*) and 8 % (most favourable year, in the absence of *S. nodorum*). *S. nodorum* developed well in the field, with leaf layers experiencing up to 60% severity. In one year a significant positive effect was found, with increased levels of *S. nodorum* in the presence of *B. graminis*. The effect on *B. graminis* in all three trials indicates an interference type competition between the two pathogens, based on the earlier arrival of *B. graminis* and that the two were developing simultaneously. However, a faster rate of development could also give an exploitation advantage to *S. nodorum*.

Data from both experiments were analysed further in a later paper (Weber, 1996), where the Lotka-Volterra model was applied, to explore the interaction between the pathogens. The original model (Eq. 3) had been modified to simulate different interaction

mechanisms, which were fitted to the disease observations. From these followed that the interaction parameter (α_{ij} ; Figure 2:4) describing effect of *S. nodorum* on *B. graminis* was higher than 1, and thus described an antagonistic effect. The effect of *B. graminis* on *S. nodorum* in Webers modified model, had the reverse sign, and the obtained value significantly larger than one, was thus used to conclude a successful modelling of the promoting effect of *B. graminis* on *S. nodorum*.

Spore production from the pathogens *P. tritici-repentis* and *P. triticina* was studied by Al-Naimi *et al.* (2005). The two species were inoculated alone or together in additive concentrations, either simultaneously or three days apart on 28-31 days old plants. Sporulation by *P. triticina* was reduced by up to 80 % in all combinations with *P. tritici-repentis* relative to when it developed alone; the effect was highest when *P. triticina* was applied at the same time or later than *P. tritici-repentis*. In the reverse relationship, *P. tritici-repentis* was less affected by *P. triticina* inoculation. When *P. triticina* was inoculated after *P. tritici-repentis*, it even promoted an increased spore production relative to when *P. tritici-repentis* was alone. In the other two combinations of inoculation time, reduction was more severe when *P. triticina* had been inoculated first. These two species are thus affected by the presence of each other, and *P. tritici-repentis* seems the stronger competitor, though it is not clear how much of the interaction effect may be ascribed to what can be expected, based on a double total density, and consequent exploitation, and how much is can be ascribed to interference competition between the two.

P. triticina and *S. tritici* were observed in a field, where both developed from natural inoculum (Chester, 1944). Due to the faster rate of growth of *S. tritici* it was observed that this pathogen removed leaves from the plant at a rate faster than what *P. triticina* could keep up with. The consequence was that *P. triticina* severity was significantly reduced. Interaction between the same two pathogens has also been assessed in a greenhouse experiment where sporulation of *P. triticina* on flag leaves was quantified in the presence of *S. tritici* (Robert *et al.*, 2004a). *P. triticina* lesions were found to be smaller and produced fewer spores in the presence of *S. tritici*, an effect which was ascribed to both direct overgrowing of the *P. triticina* lesions and indirect via the plant. The interaction has also been assessed via their effect on the photosynthetic rate of the plant (Robert *et al.*, 2004b) using the model employed by Lopes & Berger (2001) also. In this case the model was modified to accommodate a necrotrophic (A) and a biotrophic (bB) pathogen, assuming the former can overgrow the latter, but not vice versa

$$P_{x_{Abb}} \cong P_0 \times (1 - x_A)^{\beta_A} \times \left(1 - \frac{x_{bB}}{1 - x_A} \right)^{\beta_{bB}} \quad (6)$$

The results of this analysis showed no interaction between *P. triticina* and *S. tritici*, as the obtained β values found for the species alone versus together, were not significantly different.

The growth of the two fungi may overlap, but it is difficult to imagine that this is a completely neutral interaction on the leaf. The authors did observe that symptoms caused by *S. tritici* interfered with *P. triticina* development, such that the rust fungus was impaired by *S. tritici*. From these studies an interference type competition is thus evident between these two species.

P. triticina has also been studied in combination with *S. nodorum* in field trials in two consecutive years where the diseases originated from natural inoculum (Spadafora &

Cole, 1987). *P. triticina* was controlled to a range of disease levels using a specific fungicide in a range of doses. Consequent disease levels were observed to have an inverse relation to each other, which was used to conclude that competition occurred between the two. The diseases were observed in levels up to 50 and 20 % in one year and 15 and 30% the next. What type of interaction is causing the observed pattern cannot be determined, and most likely it is based on a combination of interference and exploitation competition.

Puccinia striiformis and *S. tritici* were studied by Madariaga *et al.* (1986) on 3 non-resistant wheat varieties. The first inoculation was carried out at GS 12 and the next 17 days later. Area under the disease progress curve (AUDPC) was observed from 4-14 days after the last inoculation. They observed that AUDPC for the total disease levels on two out of the 3 varieties were lower than for either of the two species of pathogens, which is taken as an indication of negative interaction. They further observed that both species were affected more severely when the other species was applied at the same time relative to a delay of 17 days.

P. hordei and *Stagonospora avenae* f.sp. *triticea* have been observed together in both a greenhouse experiment and a field trial (Shearer *et al.*, 1978). In the greenhouse either pathogen or both species were applied to a susceptible barley cultivar. *P. hordei* was applied at flowering (app GS 61) and *S. avenae* f.sp. *triticea* 7 days later, after having evaluated severity from *P. hordei*. Four concentrations of *P. hordei* and one of *S. avenae* f.sp. *triticea* were applied, excluding the controls. Seven and 14 days later necrosis was evaluated, after which sporulation by *S. avenae* f.sp. *triticea* was quantified. A positive correlation between severity of *P. hordei* and necrosis was observed, such that with increasing levels of rust more necrosis was observed. The necrosis was an effect of *S. avenae* f.sp. *triticea* development, as very little necrosis was observed in the absence of this pathogen, even at high *P. hordei* severities. In leaves infected with only *S. avenae* f.sp. *triticea* significantly less necrosis was observed, relative to in the presence of *P. hordei*. This corresponded with observed positive correlations of *S. avenae* f.sp. *triticea* spore production with both leaf rust severity and necrosis. In the field trial, diseases from natural inoculum were observed on the two uppermost leaves of susceptible varieties, and also here, a positive correlation between pycnidia production by *S. avenae* f.sp. *triticea* and infections by *P. hordei* was found.

The above section presents 8 different pathogen combinations of 11 different species, which is a somewhat more varied group than the one representing studies on biotrophic fungi. In most of the presented studies above, is it found that the biotrophic fungus is negatively influenced by the presence of the necrotrophic species, and in some cases the biotrophic promotes development of the necrotrophic species.

2.5.3 Necrotrophic-Necrotrophic

Combinations of two necrotrophic pathogens have also been the subject for a range of studies, with slightly less variety than the above group.

da Luz and Bergstrom (1987) observed *Bipolaris sorokiniana* and *P. tritici-repentis* applied to different varieties of wheat at GS 23 in the greenhouse in a replacement design, and disease severity was recorded per leaf 7 days later. Also, a sequential inoculation experiment was carried out with a range of different relative time points, again in with doses of the same total density, whether one or two species were applied. For the inoculation series, lower total disease was observed from all mixed inoculations relative to the two single species. Given that total disease severities were lower than

what would be expected based on only 50% of the total severity caused by either species when inoculated at 100 %, the interference conclusion seems valid. The findings indicate a direct interaction between the two fungi. The sequential inoculation experiments confirmed the first experiment and indicated that *B. sorokiniana* had an inhibitory effect on *P. tritici-repentis* establishment.

B. sorokiniana has also been studied in combination with *Septoria passerinii* (Morton & Peterson, 1960; Wibe & Morton, 1962). Morton (1960) found that naturally occurring levels of *S. passerinii* were lower in plots treated with *B. sorokiniana* 2 weeks before heading. The study reports 'heavy spot blotch development' from the *B. sorokiniana* application, but does not present any values for severity levels, and consequently interference and exploitation competition cannot be distinguished from this study. The pathogen combination was studied further though, by Wibe (1962) on detached leaves, where the two species were applied in various combinations of order and time intervals between events. The proportion of leaves covered with *B. sorokiniana* was found relatively unaffected by either treatments with *S. passerinii* whereas the reverse combination had significant negative effects and indicates that *B. sorokiniana* has a negative effect on *S. passerinii*.

Interaction between *P. tritici-repentis* and *S. nodorum* was studied by Adey *et al.* (1990). The experiment was designed as a replacement series with 5 relative concentrations, including the single species concentrations, which was determined such that it differed between species but resulted in approximately equal number of lesions. The study included an experiment with and without inoculation delay. In the latter, *P. tritici-repentis* was inoculated 4 days after *S. nodorum*. Pathogens were inoculated on leaves at plant anthesis (Feekes 10.5.2, GS 65). Plants were cut and let to dry, the top three leaves were collected and placed in favourable conditions for production of fruiting bodies, which were quantified. From the results the *RYT* lines were never found to be significantly higher than 1, and in the delayed inoculation experiment it was always lower than the reference *RYT* line. So there were strong indications that antagonistic effects took place between the two species and that *P. tritici-repentis* had the competitive advantage.

The two necrotrophic pathogens *Rhynchosporium secalis* and *Drechslera teres* on barley were studied in a green-house experiment by Xue and Burnett (1995) where 4 non-resistant barley varieties at GS 15 were inoculated by a replacement design, with the two species alone (5×10^3 conidia per ml) or together. Levels of disease caused by *R.secalis* was generally considerably lower in the dual inoculation, than half of the disease caused in the single species inoculations, whereas symptoms caused by *D. teres* were about half the observed in the single species application. This indicates an interference interaction, given the reduction in disease symptoms which was confirmed from an experiment with an inoculation delay of 24 hours, where the highest symptoms developed when the same pathogen was inoculated at both time points. In both combinations of the two relative to each other, *R. secalis* was more affected by the presence of *D. teres* than vice versa.

The same two pathogen species were studied under simultaneous development in a field trial, with diseases from artificial inoculation applied at GS 25 Pinnschmidt *et al.* (2002). Disease was observed for the crop several times over the growing season. Plot level severity caused by *R. secalis* and *D. teres* where applied together, were found in levels that were less than additive, relative to the levels observed alone.

These were further studied in another field trial based on artificial application, and diseases were observed on individual leaves during development in the field (Vollmer *et al.*, 2005). Disease progress on leaf layers showed a trend towards reduced levels and rates of growth when both were together. Furthermore, an association analysis of disease levels on single leaves showed that the two diseases were found more seldom on the same leaves than would be expected if they were distributed independently in the crop.

The most frequently studied combination of pathogens is that of the two wheat pathogens; *S. tritici* and *S. nodorum*, and these studies are considered in the following.

Jones and Odebunmi (1971) inoculated two spring wheat varieties at heading (Feekes 10.5, GS 58) in the greenhouse and 10 days later transferred them to a field cage. The two were applied in different proportions at the same total density (10^6 spores ml⁻¹). Disease was assessed for the whole plant once, 2 weeks after inoculation, using a score calculated based on leaf area infected per leaf and number of leaves infected per plant. The highest plant disease score was recorded where *S. nodorum* only was inoculated and the lowest where *S. tritici* was inoculated alone, and disease levels were generally decreasing with an increasing proportion of *S. tritici* in the inoculum, but *S. nodorum* was considered to be more competitive than *S. tritici*.

The *S. nodorum* and *S. tritici* combination was also studied by Harrower (1978) on three wheat varieties at the three leaf stage under controlled environmental conditions in a replacement design, across 5 relative proportions of inoculum input. Higher severities were caused by *S. nodorum* than *S. tritici*. Sporulation quantification revealed though, that spores from *S. tritici* were found in a higher amount than would be predicted from the proportional inoculum. The author used this to conclude that this species is a better sporulator than *S. nodorum*. This conclusion though, as all other replacement designs, suffers the density effects, and is not enough to conclude interaction effects between the two species on sporulation. *S. nodorum* caused higher levels of disease on leaves, almost 100% on the most susceptible cultivar with *S. tritici* causing up to 65% on the same cultivar when inoculated alone, and as seen by Jones & Odebunmi (1971) disease severities were decreasing with increasing proportions of *S. tritici* in the inoculation mixture but caused no conclusions regarding interaction between the two species on severity levels.

Jenkins and Jones (1981) carried out a range of experiments with *S. tritici* and *S. nodorum* on both spring and winter wheat. Pathogens were applied to plants grown in plots in the field. For the disease scores they found no significant effects of interaction between the two pathogens. They observed a linear relation between amount of *S. nodorum* in inoculum and leaf area with disease (10-22 % per leaf) which is used as indication for lack of interaction between pathogens. At this level of severity effects of disease treatment on grain yield/ear were additive. In another experiment, disease levels were allegedly higher, though no value was given, and single species effects on yield parameters (1000 grain weight and grain yield/ear) ranged from 23-33 % for *S. tritici* to 49 % in some treatments of *S. nodorum*. Here, levels were found to be non-additive, thus ascribable to exploitation competition.

Nolan *et al.* (1999) carried out a range of experiment on interactions between *S. tritici* and *S. nodorum* both under controlled environmental and field conditions. In the growth chamber experiment *S. tritici* and *S. nodorum*, were inoculated either alone or together in additive amounts four days apart, in either order, on the youngest leaves of 21 days old wheat plants. On these plants pathogen production was quantified in three different

ways: disease severity, ergosterol content of leaves and spores were counted. In the ergosterol analysis, the sum of levels from the single pathogen treatments were compared with levels from dual inoculation treatments, where content was quantified as a total for the two species. These comparisons are based on treatments with similar total levels of inoculum. Of the two dual inoculation treatments, significantly higher levels of ergosterol were found when *S. nodorum* was applied prior to *S. tritici* than vice versa. But for both of the combined treatments, significantly lower levels were found, compared to the sum from the individual inoculations. Disease severity per leaf was assessed after 19 days. Symptoms were distinguishable as *S. tritici* did not produce necrotic symptoms but was assessed as percentage leaf area covered by pycnidia and *S. nodorum* was assessed as percentage necrosis. *S. nodorum* produced significantly more necrosis when present alone or applied prior to *S. tritici*. Spores were counted from macerated tissue at the termination of the experiment. *S. nodorum* produced more spores in the presence of *S. tritici* compared to when it was present alone, though when the two had been inoculated together, *S. nodorum* produced more when it was present first. This was in contrast to *S. tritici* which produced the highest number of spores when alone, but when the two were present together it also produced more spores when it had been inoculated first.

In a the field trial by Nolan *et al.* (1999) *S. nodorum* and *S. tritici* were inoculated at GS 49 either alone or together, after 7 days the other pathogen was applied in the same amount and concentrations as in the single species treatments of the first date, *i.e.* an additive design. Diseases were assessed as severity on the flag leaf three times over 28 days, a period which could encompass several generations of both the two pathogens (Wiese, 1987). Lowest necrosis levels were observed in the single species inoculation treatments, with *S. nodorum* producing more than *S. tritici*. The highest levels were observed in the treatment with *S. nodorum* applied at day 0, followed by *S. tritici* a week later, a level which was significantly higher than when the had been applied together on day 0. Spores produced on 5 tillers were counted for each fungus 33 days after the first inoculation. *S. nodorum* produced the lowest number of spores when applied alone, which is in contrast to the observation that this species produced the highest number of spores when applied 7 days prior to *S. tritici*. This pathogen also produced its highest number of spores when applied prior to the other species, but single presence of the species gave higher spore levels than any of the other combinations with the other pathogen. The greenhouse versus field study gave similar results. *S. nodorum* produced significantly more spores in the presence of *S. tritici*, with the highest production when *S. nodorum* was applied first. The same behaviour was not observed for *S. tritici*, which produced the highest number of spores when inoculated alone but of the two dual inoculation treatments the highest spore counts was also recorded when *S. tritici* had been inoculated first.

In conclusion from the study by Nolan *et al.* (Nolan *et al.*, 1999), it seems that *S. tritici* promotes *S. nodorum*, while the latter pathogen has an inhibitory effect on *S. nodorum*.

Disease development from natural inoculum of the sorghum (*Sorghum bicolor*) pathogens *Colletotrichum sublineolum* and *Exserohilum turcicum* were observed together on their host in field plots over 55-65 days by Ngugi *et al.* (2001). In plots of susceptible host varieties they observed disease values up to 30 and 50 % for the two diseases developing simultaneously. The disease progress data were analysed by fitting Lotka-Volterra equations in their original form (Eq. 3), with resulting well fitting model with interaction coefficients not significantly different from zero. The non-linear regression

was based on initial conditions for estimation of model parameters, and here initial values for the regression to work with were 0.1 for the α_{ij} values. From Figure 2:4 and the discussion above though, it is evident that an α_{ij} value near zero is a wrong null-hypothesis for foliar pathogens, but rather this should be near one, due to the inherent exploitation competition for leaf area. The high disease values given above, further stress that a value of zero is unlikely. The analysis should thus have been carried out, with initial suggested values of one. The results provided by Ngugi *et al.* (2001) are therefore not adequate to draw conclusions on interaction types between the two pathogens.

From the studies of interactions between necrotrophic pathogens, the emerging pattern is less clear than in the other two combinations. The *P. tritici* – *B. sorokiniana* combination, shows that one species dominates the other, where as for the *P. hordei* – *B. graminis* combination, it seems more a matter of who gets there first. The *S. nodorum*-*S. tritici* combination, interference seems evident between the two, and *S. nodorum* is the strongest competitor and may even be stimulated by the presence of *S. tritici*.

2.6 Discussion

Interactions between foliar plant pathogens have been considered repeatedly over most of the last century, but despite a considerable amount of studies on several species simultaneous development, these studies have not been collected and discussed together. Furthermore, a general formulation of hypotheses to base analysis on has been absent. These aspects have been addressed in the present paper, where studies of interaction between species of pathogens developing on their host leaf surfaces have been collected.

The theoretical section illustrated effect of the dependence on leaf surfaces for competition between pathogens. The fundamentally important consequence is that exploitation competition (Table 2:1) always takes place, and the task is to distinguish this from other types of interaction. This implies that the two forms of interaction exploitation competition and synergism do not exclude each other, even if two species grow faster in each others presence, it is still a matter of the quickest acquiring the largest proportion of the leaf area.

The experimental section provided a presentation of studies, which have explored interaction between species of foliar fungal pathogens. Only studies that observed development on susceptible host plants were included, this was important to keep the focus on the fungal interactions and meant that observations of induced susceptibility of normally resistant host varieties by another fungal species were not considered.

From the studies, it is evident, that some form of competition is the dominant form of interaction, where in some cases it is difficult to establish whether this is through interference or exploitation, but synergism was observed also.

The trophic interaction of the fungi with the host plant seems to play an important role in determining the type of interaction. In the studies of two biotrophic species, only *P. hordei* and *B. graminis* f.sp. *hordei* were represented. Between these two species the interaction outcome was generally decided by the first arriving pathogen, and thus there is not one species that seems superior to the other. In studies of interaction between a biotrophic and necrotrophic species the majority of studies found that the necrotrophic fungus had a negative influence on the biotrophic species and in some cases the biotrophic species promoted development of the necrotrophic species. In the necrotrophic interactions studies negative interactions were generally observed, though

one species may be superior to the other or the relation is more even, but still with an advantage of arriving early, such that it is a matter of who arrives first.

Synergism generally occurs in combinations where a biotrophic species promote the development of necrotrophic species, as in the relation between *P. hordei* and *S. avenae* f.sp. *triticea* (Shearer *et al.*, 1978) and *B. graminis* and *S. nodorum* (Weber *et al.*, 1994). The biotrophic fungi depend on living host cells for establishment, growth and sporulation while the necrotrophic fungi kill host cells and is able to derive energy from dead host material (Oliver & Ipcho, 2004). The mechanism behind this interaction is probably related to the accumulation of nutrients by the biotrophic species, which are also beneficial to the necrotrophic fungi and thus promotes its development.

Density dependence is important for elucidating interspecific interaction, when data are based on different interspecific inoculum doses, as was illustrated with the discussion of the replacement versus additive design. The relative doses based on which species behaviour is compared, influences the interspecific response, and comparing behaviour from two doses, in the presence and absence of a competitor means that intraspecific interactions may be confused with interspecific ones. This problem was present in several of the studies presented above, where species were studied in replacement designs (da Luz & Bergstrom, 1987; Adey *et al.*, 1990; Xue & Burnett, 1995; Al-Naimi *et al.*, 2005). This problem is evident whether comparing visual disease levels, including consideration of the virtual lesion size, or when disease growth is analysed by applying the Lotka-Volterra model.

The leaf as a finite and fundamental resource has implications for the application of the Lotka-Volterra model. The model includes the term *K*, carrying capacity or maximum disease severity, which controls development of the modelled competitors. Applying this model to foliar diseases must be done with caution. Fundamentally, the expected behaviour in the absence of any interaction, which is not exploitation competition, must be clearly formulated. This consideration had not been included in the analysis by Ngugi *et al* (2001), and no conclusions could therefore be drawn from the analysis. Modifications are required, such as avoiding negative growth as done by Weber (1996).

The relative timing of applying the two pathogens was important for the fungal interaction in several cases. This may be due to both effects via the plant, in establishing defence reactions, and directly between the fungi. Some species may require a period for establishment during which they are more sensitive to other fungi, *e.g.* during germination and spore production. Necrotrophic fungi produce toxins (Oliver & Ipcho, 2004), which could influence co-existing species, but given that the importance of timing has been observed for all combinations of trophic host interaction, this does not alone explain the effect. Olesen (2005) found that the interaction between *B. graminis* f.sp. *hordei* and *P. hordei* was mediated through the plant, and likely a range of factors are involved in these types of interactions.

To able to develop knowledge on interactions between foliar fungal pathogens ultimately into recommendations for agricultural management, the importance of these interactions in the field must be established. The information may then be used either in variety choice, *e.g.* some types of resistance could be more important to combine than others and in integrated control strategies, where threshold control levels are determined based on the combination of co-occurring pathogens.

The majority of the above studies have been carried out in the greenhouse, which offers a very different environment both to the fungi and their host plant, relative to the field. The

greenhouse is characterised by its constant environmental parameters such as temperature and humidity, whereas in the field temperature and moisture vary with climatic conditions, and further wind, nutrient variability, weeds and other microorganisms impact both the plant and fungi. The consequences of these differences can affect both the ability of the pathogen to develop as well as have impact on the state of the plant and its host-properties. Greenhouse experiments are suitable for preliminary investigations on basic biological mechanisms related to pathogen interactions, but conclusions regarding interactions between naturally developing species should not be drawn without inclusion of outdoor field trials. Several presented studies above report stronger interactions in the greenhouse versus field experiment, thus the significance of an interaction observed in the greenhouse for disease levels in the field must be re-established in this environment.

Disease levels in most experimental trials are made to focus on the potential interaction, to determine if a specific biological relationship exists, which may promote inoculation in high doses to ensure disease establishment. Further, the experimental inoculations occur as one or a few events, where all spores are deposited on the plant surface at one time point. This may have consequences for immediate density effects on the fungal establishment, but also may influence the response of the plant to the pathogen. The contrast to this approach is to observe diseases in the field, preferably developing from natural inoculum. It was found by Vollmer *et al.* (2005) that the two pathogens *R. secalis* and *D. teres* were negatively associated on single leaves in the field. This trial was based on artificial inoculum which had been applied at GS 13-25, and the observation was on higher leaves, to where the pathogens had dispersed independently. These types of interactions are easily missed in experiments with high inoculation doses. Determination of natural inoculum levels could be associated with spore trapping in an around the canopy, to determine natural levels of spore availability.

An objective behind accumulating the literature on a given topic is to use this for drawing generalisations and thereby be able to formulate even better hypotheses for future studies. An important question then is: to what extent are the existing papers representative for actual experimental work carried out. In the present context, where people most often have aimed to establish an antagonistic or synergistic effect it may be likely that a degree of publication bias is inherent, such that where people have set out to establish either of these interaction types but results caused a rejection of the hypothesis, the studies are more likely not to have received publication.

To increase the knowledge of the importance of pathogen interactions in the field, future focus should be on exploring natural pathogen developments in the field more so than further greenhouse studies, preferably based on natural inoculum. Monitoring occurrence and distributions of diseases at different spatial levels of crops, such as between fields, spatial distribution within fields, on individual plants and single leaves, can be analysed by statistical independence tests to increase understanding of natural dynamics of co-existing pathogens. This type of information should be collected with close reference to relative resistance properties of the host plant towards pathogens in focus. This type of information will increase our basic understanding on how different pathogens are naturally distributed, and from this derive hypotheses on antagonistic or synergistic interactions

In a world with increasing pressure, from politicians as well as those generally concerned with the natural environment, to reduce input of pesticides and increasing the areas

allocated to low-input farming practices, there is good reason to focus on improving our understanding of biological processes in crops.

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2.8 Tables

Table 2:1 Different types of interaction between two species (A and B)

<i>Interaction type^a</i>	<i>Effect on A^b</i>	<i>Effect on B</i>	<i>Other terms^c</i>
<i>Indifference</i>	<i>0</i>	<i>0</i>	<i>No interaction</i>
<i>Commensalism</i>	<i>0</i>	<i>+</i>	
<i>Ammensalism</i>	<i>0</i>	<i>-</i>	
<i>Mutualism</i>	<i>+</i>	<i>+</i>	<i>Synergism</i>
<i>Predator – prey</i>	<i>+</i>	<i>-</i>	
<i>Competition</i>	<i>-</i>	<i>-</i>	
<i>Exploitation</i>	<i>Indirect inhibition, short supply of a common resource (leaf area)</i>		<i>Density dependence</i>
<i>Interference</i>	<i>Direct inhibition, actively inhibit between the two species</i>		<i>Antagonism</i>

^a from Christiansen & Fenchel (1977).

^b '0': no effect, '+' : a positive effect, '-' : a negative effect.

^c These terms are those generally found in the plant pathological literature.

Table 2:2 Change in fungal species names from original literature. The table presents the name used in the text and the names used in the original literature.

Name used in text	Previous name, as used in reference	References with original use
<i>Blumeria graminis</i>	<i>Erysiphe graminis</i>	Simkin & Wheeler, 1974; Kiessling & Hoffman, 1985a; Kiessling & Hoffman, 1985b
<i>Bipolaris sorokiniana</i>	<i>Helminthosporium sorokinianum</i> ; <i>H. sativum</i> , <i>Drechslera sorokiniana</i>	Morton & Peterson, 1960; Dickinson & Skidmore, 1976
<i>Puccinia triticina</i>	<i>Puccinia recondita</i> f.sp. <i>triticina</i>	Van der Wal <i>et al.</i> , 1970; Spadafora & Cole, 1987
<i>Stagonospora nodorum</i>	<i>Septoria nodorum</i> ; <i>Leptosphaeria nodorum</i>	Jones & Odebunmi, 1971; Harrower, 1978; Jenkins & Jones, 1981; Spadafora & Cole, 1987; Adey <i>et al.</i> , 1990
<i>Stagonospora avenae</i> f.sp. <i>triticea</i>	<i>Septoria avenae</i> f.sp. <i>triticea</i>	Shearer <i>et al.</i> , 1978

Table 2:3 Dynamic outcomes of the Lotka-Volterra model (Begon *et al.*, 1996)

	$K_B/\alpha_{AB} > K_B$	$K_B/\alpha_{AB} < K_B$
$K_A/\alpha_{BA} \geq K_B$	Stable equilibrium	A wins
$K_A/\alpha_{BA} < K_B$	B wins	Depends on initial conditions

Table 2:4 Pathogens studies sorted by host species

<i>Pathogen</i>	Pathogen	Gen. Studied^a	Exp. Cond.^b	Reference
Barley (<i>Hordeum vulgare</i>)				
<i>Bipolaris sorokiniana</i>	<i>Septoria passerinii</i>	1(+) ^c	F	Morton & Peterson (1960)
<i>B. sorokiniana</i>	<i>S. passerinii</i>	1	C	Wibe & Morton (1962)
<i>Blumeria graminis</i> f.sp. <i>hordei</i>	<i>Puccinia hordei</i>	1,1+	C, F	Simkin & Wheeler (1974)
<i>B. graminis</i> f.sp. <i>hordei</i>	<i>P. hordei</i>	1	C	Round & Wheeler (1978)
<i>B. graminis</i> f.sp. <i>hordei</i>	<i>P. hordei</i>	1	C	Kiessling & Hoffman (1985a; 1985b)
<i>B. graminis</i> f.sp. <i>hordei</i>	<i>P. hordei</i>	1	C	Olesen (2005)
<i>Drechslera teres</i>	<i>Rhynchosporium secalis</i>	1	C	Xue & Burnett (1995)
<i>D. teres</i>	<i>R. secalis</i>	1+	F	Pinnschmidt <i>et al.</i> (2002)
<i>D. teres</i>	<i>R. secalis</i>	1+	F	Vollmer <i>et al.</i> (2005)
<i>Puccinia hordei</i>	<i>Stagonospora avenae</i> f.sp. <i>triticea</i>	1+	F	Shearer <i>et al.</i> (1978)
Bean (<i>Phaseolus vulgaris</i>)				
<i>Colletotricum lindemuthianum</i>	<i>Uromyces appendiculatus</i>	1	C,	Lopes & Berger (2001)
<i>Phaeoisariopsis griseola</i>	<i>Uromyces appendiculatus</i>	1+	F	de Jesus <i>et al.</i> (2001a) de Jesus <i>et al.</i> (2001b)
Sorghum (<i>Sorghum bicolor</i>)				
<i>Colletotrichum sublineolum</i>	<i>Exserohilum turcicum</i>	1+	F	Ngugi <i>et al.</i> (2001)

Continued on next page

<i>Pathogen</i>	Pathogen	Gen. Studied^a	Exp. Cond.^b	Reference
Wheat (<i>Triticum aestivum</i>)				
<i>Blumeria graminis</i>	<i>Stagonospora nodorum</i>	1+, 1+	C, F	Weber <i>et al.</i> (1994); Weber(1996)
<i>Bipolaris sorokiniana</i>	<i>P. tritici-repentis</i>	1	C	da Luz & Bergstrom (1987)
<i>Puccinia striiformis</i>	<i>Septoria tritici</i>	1	C	Madariaga & Scharen (1986)
<i>Puccinia triticina</i>	<i>P. tritici-repentis</i>	1	C	Al-Naimi <i>et al.</i> (2005)
<i>P. triticina</i>	<i>Septoria tritici</i>	1+	F	Chester (1944)
<i>P. triticina</i>	<i>S. tritici</i>	1	C	Robert <i>et al.</i> (2004a)
<i>P. triticina</i>	<i>S. tritici</i>	1, 1+	C, F	Robert <i>et al.</i> (2004b)
<i>P. triticina</i>	<i>Stagonospora nodorum</i>	1+	F	Spadafora & Cole(1987)
<i>Pyrenophora tritici-repentis</i>	<i>S. nodorum</i>	1	C	Adee et al . (1990)
<i>Seproria tritici</i>	<i>S. nodorum</i>	1	SF	Jones & Odebunmi (1971)
<i>S. tritici</i>	<i>S. nodorum</i>	1	C	Harrower (1978)
<i>S. tritici</i>	<i>S. nodorum</i>	1, 1+	SF, F	Jenkins & Jones (1981)
<i>S. tritici</i>	<i>S. nodorum</i>	1, 1(+) ^d	C, F	Nolan <i>et al.</i> (1999)

^aGenerations studied (one (1) or several (1+)). This is in some cases difficult to establish as measurements are recorded at a time after inoculation, where one generation in principle could have been completed. In cases where measurements are taken at inoculated single leaves and generations are not mentioned, the study is reported as 1.

^bExperimental condition refers to controlled (C; green house), field (F), semi-field (SF; outdoors in pots)

^c*S. passerinii* developed from natural inoculum, and thus over an unknown number of generations, while *B. sorokiniana* was inoculated 2 weeks before heading.

^dIt is not clear whether several generations were important for disease development in the field experiment

2.9 Figures

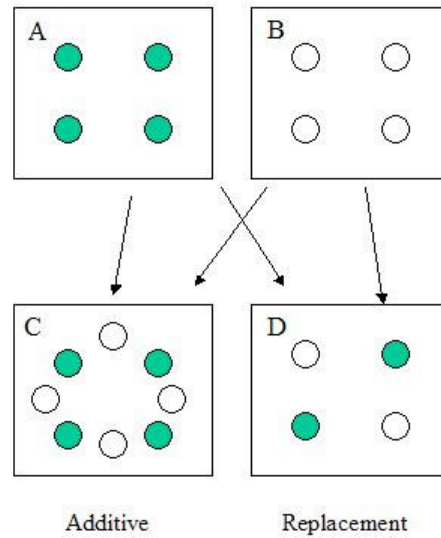


Figure 2:1 Replacement versus additive designs; two fundamental designs to explore interaction between two species or pathotypes by. Comparing the results from observing the two alone (A and B) and together (C and D), either under constant individual densities (Additive design, C) or a constant total density (Replacement design, D). Redrawn from Snaydon (1991).

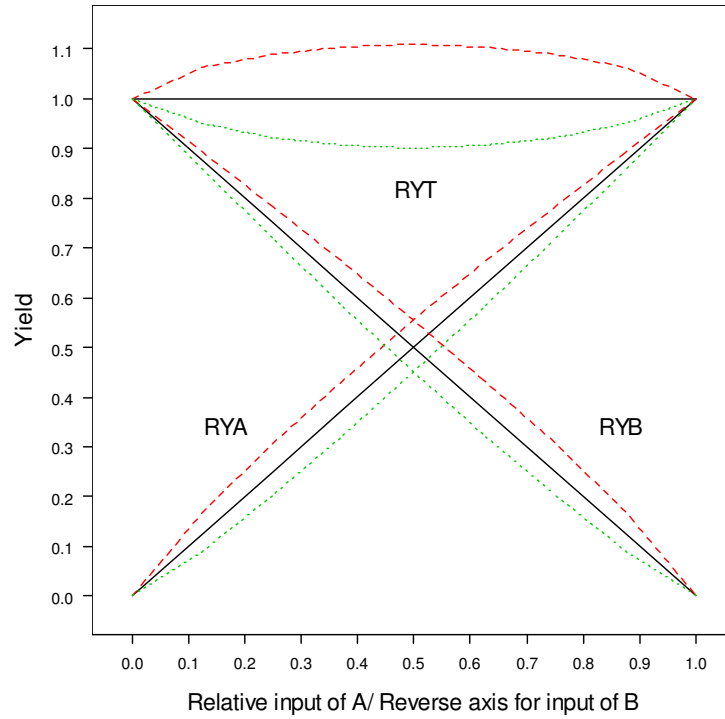


Figure 2:2 de Wit curves. The x-axis represents input proportion of species A the reverse axis (not drawn) is the input proportion of species B. The yield observations of A and B at the various input proportions are presented relative to their yield when observed alone (RY_A and RY_B) The relative yield total (RYT) is the sum of the two at the range of input proportions. The analysis compares the observed values to the straight null lines, which correspond to a situation with equal inter and intra specific competition. From this model observed lines below the null-lines indicate a stronger inter than intra-specific competition and observed lines above the null lines indicate that intraspecific competition is stronger than inter.

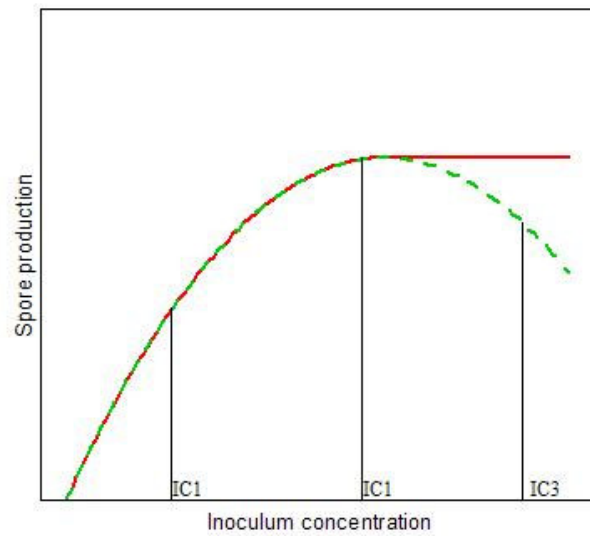


Figure 2:3 Relation between response of a trait and intraspecific density dependence, e.g. spore production with inoculum density. The response can have different shapes; here asymptotic (full line) and quadratic (broken line) are shown. Making observations at IC1, IC2 will give different results, and at IC3 they depend on the response curve

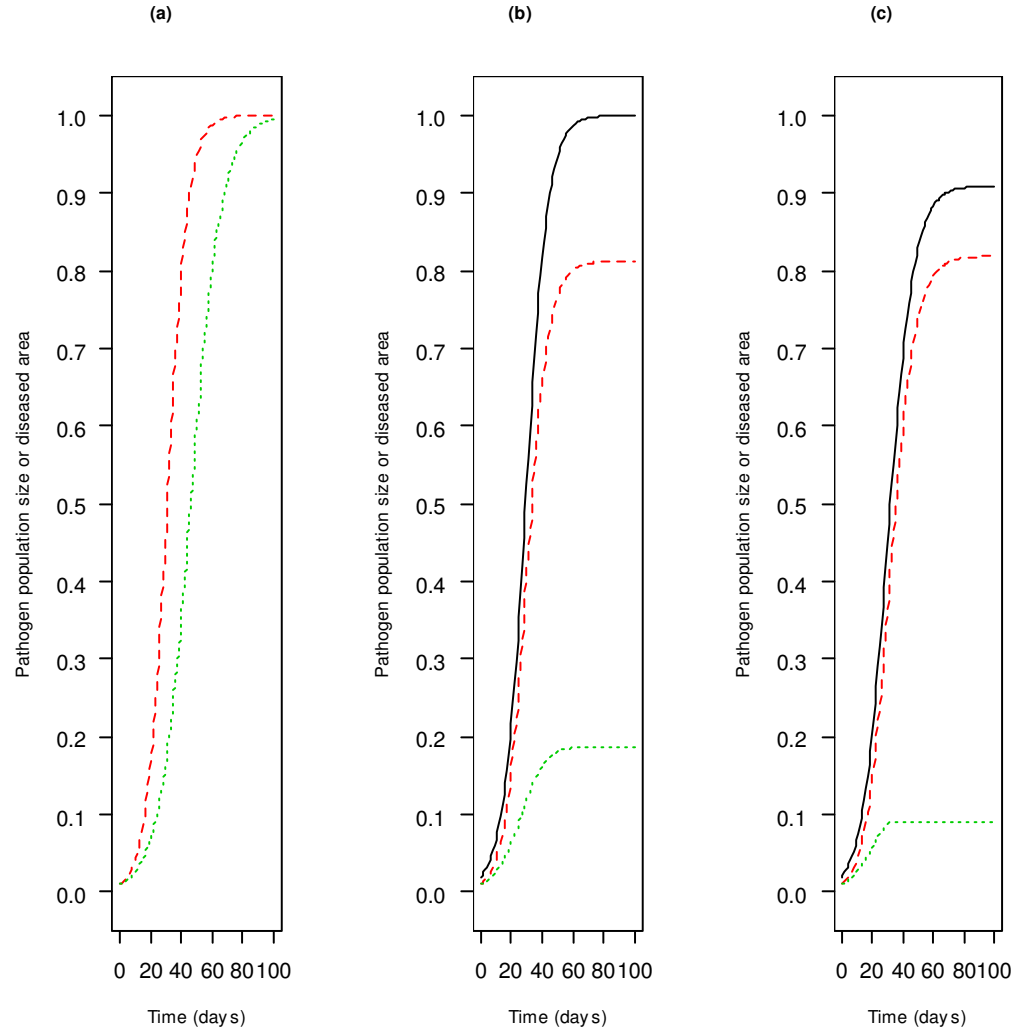


Figure 2:4. Effects of different kinds of interaction on disease levels. a) a logistic model (Eq. 1) for the two species A and B developing on separate leaves or the Lotka-Volterra model (Eq. 3) with $\alpha_{ij}=0$. b) The same model parameters but simultaneously on the same leaf (Eq. 2) or Lotka-Volterra model with $\alpha_{ij}=1$. c) Antagonism, the Lotka-Volterra model (Eq. 3) with $\alpha_{ij}=2$. All graphs have $K_A = K_B = 1$, $r_A = 0.15$, $r_B = 0.20$

3 Simultaneous epidemic development of scald (*Rhynchosporium secalis*) and net blotch (*Drechslera teres*) on individual leaf layers of a spring barley crop

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3.1 Abstract

Foliar pathogen species are commonly found developing simultaneously in a field, and interaction between species has been shown to affect total disease level. In the present study, a field trial with application of *R. secalis* (causing scald) and *D. teres* (causing net blotch) was conducted with three spring barley varieties. Each pathogen was applied alone as well as in combination with the other one, either at the same time or 26 days apart. Disease severity was assessed during the season on individual leaves; net blotch severity reached up to 35% and scald severity 20% on individual leaves. A negative association between scald and net blotch severity was found on individual leaves for several combinations of leaf layer and variety indicating antagonistic effects between the two diseases in the field. Further, maximum disease levels of either disease were often lower on individual leaf layers when both pathogens had been inoculated. These effects were not so strong though, as to be evidenced as non-additive effects of the two pathogens on total disease level. Disease development was described by fitting an exponential model to severity data over time for each leaf layer per variety and treatment. Parameters of the exponential model showed similar patterns as maximum disease severity but with fewer significant effects. The results emphasize the epidemiological relevance of interactions between pathogens in the field and of considering leaf layer-specific dynamics of crop growth and disease development.

3.2 Additional Keywords

Multiple disease interactions, disease association, disease model, leaf senescence, Kendalls test.

3.3 Introduction

It is a basic ecological assumption, that two species exploiting the same resource have the potential to affect each other (Begon *et al.*, 1996). Several studies which have dealt

with multiple diseases have reported interaction between these (Madariaga & Scharen, 1986; Xue & Burnett, 1995; Nolan *et al.*, 1999). These interactions must be understood as they have potential to influence dynamics of multiple diseases in the field, their severity levels and yield effects (Savary & Zadoks, 1992; Savary *et al.*, 1997; Savary *et al.*, 2000). Multiple diseases may have severe economical implications (Blackshaw, 1986)

Two general types of interspecific interactions between pathogens may be distinguished with respect to their effects on disease levels: antagonism where one pathogen has a negative effect on disease levels of the other and synergism where one pathogen promotes disease development of the other. Such effects may be due to different interaction mechanisms such as competition for space or nutrients, altered host susceptibility via induced resistance, or toxin production by one pathogen suppressing the development of the other. Foliar pathogens all depend on the availability of leaf area. Density dependence, the regulatory process reducing the growth rate of populations as their densities increase in relation to the available resources (Begon *et al.*, 1996), must therefore be taken into account when considering interaction.

The crop is often considered as a homogeneous area in disease assessment, i. e., disease is assessed as percentage of the visible leaf area. In reality, however, the host is a growing resource mainly defined by the dynamics caused by growth and senescence of leaf layers. The substrate for disease growth thus comprises discrete leaf areas that emerge and are removed in time and space. Leaf removal also removes disease requiring the pathogen to disperse vertically in the canopy and follow plant development to maintain itself. Field disease assessments usually regard disease as total disease on present leaf layers, thus ignoring that the individual leaf is the physical unit of resources available for the development of foliar pathogens. Physical interactions between pathogens are expected to occur on individual leaves and these must be observed if the role of pathogen interactions for epidemiological processes and dynamics is to be understood.

Research related to plant disease epidemiology and crop protection often relies on variables indicating the overall disease severity level of a particular observational unit, such as the area under the disease progress curve (AUDPC; Shaner & Finney, 1977) or the maximum disease severity observed over time. The latter will often correspond to the last observation in time, although in some cases, removal of severely diseased leaves will cause a disease decline and observations from later dates will thus show disease severity values below the previously observed maximum. In cases where highly diseased leaves have been removed, the maximum disease severity across observation dates may thus be the preferred variable to indicate the overall disease level of an observational unit.

The shape of the disease progress curve is another descriptor of disease development. It is typically described based on a mathematical model for growth and parameters from the model may then be compared between treatments. Disease progress on plants has been described by a range of models (Jeger, 2004). They are usually applied at the crop level, i.e., the models describe the sum of the disease developing on different parts or layers of the canopy (Jeger & Viljanen-Rollinson, 2001, and references therein) . These models often consider density , the logistic model is an example. Studies describing disease development on individual leaf layers have used exponential (Beresford & Royle, 1991) and logistic models (Østergård & Pons, 1996; Young *et al.*, 2003; Pons-Kühnemann, 2005).

The time of arrival on the plant of two potential pathogens is an important issue. The primary inoculum of two pathogens is unlikely to arrive at the same time under field conditions, but rather over a period defined by the presence of inoculum sources and climatic conditions. A time gap could have implications with respect to the establishment of the later arriving species if the first arriving pathogen causes modifications of the host as a substrate to the second pathogen other than simple occupying space.

Scald (caused by *Rhynchosporium secalis*) and net blotch (caused by *Drechslera teres*) are both important barley diseases in the temperate humid world and they occur together in the field. The dynamics of their interactions are here explored in the field over several consecutive pathogen generations to gain a better understanding of their importance for the disease dynamics of either pathogen. Greenhouse trials have shown that scald and net blotch significantly affect each other within a single generation following inoculation (Xue & Burnett, 1995). Field disease assessments of whole plots indicated the same (Pinnschmidt *et al.*, 2002). This study presents an approach to observe disease development on individual leaves under field conditions. The two pathogens are applied to the crop alone and in different combinations over time and disease is assessed at individual leaves. Association between diseases on leaves, the maximum disease severity as well as parameters of the exponential growth model are analysed.

3.4 Materials and Methods

3.4.1 The crop

Three spring barley varieties were grown in a field trial in 2003 at the Danish Institute of Agricultural Sciences, Research Centre Flakkebjerg, to obtain data on simultaneously developing field epidemics of scald and net blotch. Varieties differing in their susceptibility towards the two pathogens were chosen: Brazil (relatively susceptible to both pathogens), Goldie (more susceptible to net blotch than to scald) and Punto (more susceptible to scald than to net blotch). All three varieties have a relatively low susceptibility to leaf rust and powdery mildew (Anonymous, 2000; Anonymous, 2001; Anonymous, 2002)

Plots of 2.5×2.5 m spring barley were surrounded by 5 m oat (*Avena sativa*). The trial consisted of three replications with a random distribution of varieties and treatments within replication. The trial was sown on 8 April 2003 with a density of 350 plants / m². Fertiliser was applied as pig slurry in a level corresponding to 60% of the local N recommendation from the Danish Plant Directorate. The trial received a blind harrowing on 22 April, prior to plant emergence and a herbicide application on 27 May using 1½ L MCPA / ha. There was abundant rain throughout the season and, therefore, a constantly high natural humidity level.

3.4.2 Pathogen treatments

The pathogens were applied either alone or with the other pathogen and across two time points, with the second inoculation 26 days after the first (Table 3:1). A non-inoculated control was included, giving a total of six treatments and hence a total of 54 field plots. The early inoculation was made at Zadoks' growth stage 13 (GS; Zadoks *et al.*, 1974) and the late inoculation treatment was carried out at GS 25.

Drechslera teres

The early inoculation with *Drechslera teres* at GS 13 was made by applying infected straw which had been collected from a range of sources in previous years. Wheat straw was used for all plots not treated with early *Drechslera teres* to have an equal effect of the physical presence of the straw in the trial. The straw was cut in a compost-grinder and mixed to create a homogenous mixture. In each plot 750-800 g straw were distributed on the day of the early inoculation (2 May). The straw was carefully put on the ground to avoid its spread by wind. Wind speeds were relatively low on that day with an average of 3.9 m/s. A precipitation of 3.8 mm during the following night helped to keep the straw to the ground.

The late *Drechslera teres* inoculation was done using a spore suspension. The initial material for spore production was taken from frozen spring barley leaves with distinct net blotch lesions collected from a range of locations in Denmark. Spores were produced by growing the fungus on agar and on live plants.

Grass agar plates (filtrate of 32.5 g/liter of boiled clover-rich grass fodder pills for cattle and 20 g/liter of Bacto Agar) were used as substrate as this had been reported to allow high sporulation (Møller, 1992). The agar plates were incubated at 12 h light and 12 h darkness and 22°C. Spores were washed off the plates using demineralised water. The spore suspension was then kept at -20°C until it was used.

For the production of spores on plants, highly susceptible spring barley varieties were sprayed with a spore suspension at the 2-3 leaves stage, and covered with a fitting transparent plastic lid to maintain a high humidity. Plants were then kept in the greenhouse for 17 days. Lesion bearing leaves were cut and dried. The dried leaves were placed in humid chambers at 17°C and darkness for 3 days to induce sporulation. Thereafter, they were washed with demineralised water to obtain a spore suspension. From both spore production methods, a sample was taken to determine the spore concentrations prior to freezing of the suspension.

For inoculations, spores produced on agar were mixed with spores produced on plants.

At the day of inoculation, suspensions were taken from the freezer in the morning and thawed at room temperature. Approximately 15 l spore suspension and a few drops of Tween 20 were put into a knapsack sprayer just before going to the field. While in the field the knapsack sprayer was repeatedly shaken to ensure a uniform spore suspension. Plots were inoculated on 28 May by walking around them and spraying the solution ensuring an even distribution of inoculum across plots. The spore suspensions were applied in the early evening when wind speed was low to reduce drift of spores. The inoculation was repeated after two days to ensure the successful establishment of the pathogen in the crop.

The late inoculations of *Drechslera teres* contained about 1.5×10^5 spores/m², but the inoculum also contained a large amount of mycelia from the agar plates which may infect plants.

Rhynchosporium secalis

Isolates of *Rhynchosporium secalis* collected in 2002 from different Danish locations were grown on Lima Bean Agar and maintained under 24 h darkness at room temperature for 3-4 weeks. Spores were washed off the plates using sterile water.

Samples were taken from each suspension for determining spore concentrations. The suspensions were then placed at -20°C until further use. The inoculation procedure was similar to the late *Drechslera teres* application. *Rhynchosporium secalis* was applied to the plants using 1.5×10^6 spores/m².

3.4.3 Disease Assessment

To determine whether either of the pathogens entered the trial via seeds, three plots of each variety were checked thoroughly for signs of diseases on 8 May, before the artificial inoculation would have given rise to infections, but at a time when seed borne would have done so.

To follow disease epidemics in detail, plants were sampled in the field for assessment of disease on individual leaves. Sampling from the three replicates in the field was not possible within a single day, which means that disease samples were taken from different replicates at different stages in their epidemic development, but all samples from one replicate were collected on the same date (Table 3:2). Nine plants were selected randomly from each plot and taken back to the laboratory where leaves of the main tiller were pulled off and attached to plain white paper using transparent sticky tape. Each sheet of paper contained up to 9 leaves of the same leaf layer. The variety Brazil grows eight and Goldie and Punto grow nine leaf layers. The papers with leaves were piled up, with filter paper between sheets and compressed by heavy weights. They were left like this until leaves were dry.

Assessments included disease severities (necrosis) and senescence as percentage of leaf coverage. The variables recorded were: senescence, defined as brown and dry leaf tissue developing from the tip towards the base, scald and net blotch severity, recorded as percentage area of leaf covered by lesions caused by *Rhynchosporium secalis* and *Drechslera teres* respectively. Chlorosis was not evident as part of the net blotch symptoms on any of the varieties. Generally few other diseases were observed, observations of such were therefore simply recorded into one category, 'other disease', along with disease symptoms which could not be clearly identified and symptoms where there was uncertainty about the disease. Total disease is calculated as the sum of severities of scald, net blotch and other disease.

The three different types of disease data (scald severity, net blotch severity and total disease) are hereafter referred to collectively as disease measures.

Assessments are only included from fully developed leaves. Symptoms of different diseases do not overlap, whereas disease and senescence does, the result of which is, that disease severity and the senescent area may add to more than 100%. The decision to assess disease severity from the total leaf area, rather than only the green leaf area, was made to make values comparable between dates as absolute green leaf area changes with increasing senescence.

When assessment of all leaves had been completed, approximately 130 leaves were selected randomly, across treatments and severity levels, and assessed again to test the precision of the assessments.

3.4.4 Data Analysis

The analysis of the disease severity data on the single leaves included analysis of association between disease severities of the two pathogens on individual leaves, maximum disease severity of disease measures, and progress curves for the two diseases. In the following the term leaf layer sample is used for the up to nine observations made

on a leaf layer within variety, treatment, replication and date. The development of disease on a leaf layer within variety, treatment and replication is considered a leaf layer epidemic and the three replicates as epidemic replicates

The natural dynamics of the leaf development led to the fact that different leaf layers have been observed at different points in time and a different number of times, ranging between 2 and 4.

The three layers below the flag leaf (F-1, F-2, F-3) used in the analysis all have been observed more than three times for all leaf layer epidemics across replicates. Thus a total of 162 leaf layer epidemics were included. Observations from the flag leaf were not included, as the flag leaf represents a relatively insignificant proportion of the canopy in spring barley.

All leaf layers will be included in an analysis elsewhere on the leaf – approach versus the crop level approach in disease assessment.

Analyses were done using R (R Development Core Team, 2005).

3.4.5 Leaf senescence subsets

If leaves with high levels of senescence are removed from the analysis disease observations are taken out also. To be able to discuss the effect of this, subsets of leaf layer samples were made based on senescence levels: 'Senescence subset x' contained leaves with < x% senescence. Three subsets were made, for x=10, 50 and 100 % respectively.

3.4.6 Disease associations

Association between the disease severities observed on individual leaves was evaluated using Kendalls coefficient of rank correlation, τ (Hollander & Wolfe , 1999). This analysis tests if the severity levels of the two diseases on individual leaves are correlated. Correlations were computed within leaf layers only, to ensure a similar history with respect to time, inoculation and climate. Only observations from treatment 4, where both pathogens were inoculated together, for data from senescence subset 50 and the last three sampling dates were used. Leaf layer samples with at least 5 observations of both diseases were included in the analysis

3.4.7 Maximum disease severity

The maximum disease severity was used to describe the disease level in a plot. The mean severity value of net blotch, scald and total disease was determined for each leaf layer sample. The maximum of the resulting values over time represents the maximum disease severity of the respective disease for each leaf layer epidemic. It is hereafter referred to as maximum severity for the respective disease measures. The maximum severity for total disease of a given leaf layer epidemic is not necessarily the exact sum of the two individual diseases. This so, as the three disease measures for the given leaf layer epidemic may either not originate from the same date within a leaf layer epidemic and the category with other diseases and unidentified symptoms is included here also.

Maximum severity data were third root transformed prior to analysis to improve homogeneity of variances. Analysis of y_{max} values was made for all three senescence subsets

3.4.8 Progress curves

An exponential growth curve was fitted to the data for each leaf layer epidemic:

$$y = y_0 \times e^{rt} \quad (1)$$

y_0 is the disease severity of a leaf layer epidemic at age t of the leaf layer, y_0 is the amount of disease at time 0, and r is the leaf layer specific intrinsic rate of disease increase. Leaf age was assumed for each layer per variety as the time elapsed since leaf emergence. Date of emergence was determined as the median time point between no leaf and a fully expanded leaf. Exponential regressions were only computed for data from senescence subset 50 and for leaf layer epidemics with data from at least 3 observation dates, which accounts to 157 leaf layer epidemics. For leaf layer epidemics where no or very little disease had developed, r and y_0 were set to zero, i.e. when a maximum of three non-zero observations were made. This applied to 17 cases for net blotch and 90 for scald.

The parameters were estimated for the remaining leaf layer epidemics. For these there were some cases where negative growth rates (r) were estimated (6 for net blotch and 10 for scald). These growth rates were replaced by zero and the y_0 was replaced by the average observed disease severity for that specific leaf layer epidemic.

The exponential growth model did not fit 5 respectively 2 leaf layer epidemics of the two diseases. The outcome was a total of 152 sets of parameters for net blotch and 155 for scald.

3.4.9 Analyses of treatment subsets

Analyses of variance were used to compare maximum severity and parameter estimates from the exponential regressions. Different ANOVA models were considered. The models below include data from all varieties and leaf layers but from different subsets of treatments (Table 3:1). In the model formulations a is the dependent variable (y_{max} , r or y_0) and variety, layer and treatment factors (treatA, Dt, Rs, treatT: see below) are the independent variables. For all models replication was treated as random effect.

I. One model included the complete dataset of leaf layer epidemics and hence data from all six pathogen treatments (treatA). This is referred to as the full mode

$$a \sim \text{treatA} * \text{layer} * \text{variety} \quad (2)$$

II. For total disease, additive effects of inoculation of the two pathogens are tested by comparing results on total disease levels when only one or both pathogens have been inoculated. The treatment factor is written as two new factors given by the pathogen applications, Dt and Rs, with values 'no' and 'early' (Table 3:1). This analysis includes only data from treatment 1-4 to obtain balance in the test. The analysis was performed for data from all varieties in the analysis and within each variety. This model is referred to as the interaction model.

$$a \sim \text{Dt} * \text{Rs} * \text{layer} * \text{variety} \quad (3)$$

III. The third model compares developments of either of the two individual diseases from the early inoculation under the three different inoculations of the other disease (non, early, late). This analysis evaluates effect of the other pathogen, including its timing of application. The analysis included data from treatments (treatT) 3, 4, 6 for net blotch and 2, 4, 5 for scald. This model is referred to as the time model.

$$a \sim \text{treatT} * \text{layer} * \text{variety} \quad (4)$$

3.5 Results

No disease symptoms were observed during the 1st field check on May 8, indicating that seed-borne transmission of *R. secalis* and *D. teres* was negligible.

All inoculations lead to establishment of disease.

A good agreement between the two independent assessments of single leaves indicated that the disease assessments were repeatable and sufficiently precise (data not shown).

Net blotch was observed throughout the trial but in higher severity levels where *D. teres* had been applied (treatment 3-6, Table 3:3). Net blotch severity on individual leaves reached levels of up to 35 % (data not shown) in the plots that experienced the highest disease levels (Goldie with early application of *D. teres*, treatment 3, 4 and 6). But even in the non-treated plots levels up to 10 % were observed (data not shown). Net blotch reached the highest disease levels seen in the trial on the variety Goldie, followed by Brazil. Net blotch was observed on all leaf layers of any variety.

Scald was generally only observed where *R. secalis* had been applied. The scald severity ranged from 0 to 20 % on single leaves with generally lower severities on upper leaves (F-1, F-2). The highest levels of scald were observed on Punto, followed by Brazil. There were leaf layers with no observations of scald, and hence a maximum severity of zero (Table 3:3).

Total disease is the sum of scald, net blotch and observations of other diseases. Observations in the category other diseases, were made for almost all leaf layer samples, generally in levels below 5 %, except for Brazil which had some observations above this level (6% of the observations above zero were higher than 5%). This means that there are some cases where total disease was markedly higher than the sum of the maximum severities of the two single diseases (Table 3:3).

3.5.1 Disease associations

Only negative associations were found between severities of the two diseases on individual leaves in the Kendalls association analysis. Significant negative associations were found on all varieties on either layer F-2 or F-3, of which four were significant out of a total of seven (Figure 3:1). The association analysis was not possible in two cases, leaf layer F-1 and F-2 on Goldie, as less than 5 observations of scald were made here.

3.5.2 Maximum disease severity and disease development

For the two individual disease measures, all three factors (treatment, leaf layer and variety) had major influence in determining the maximum level of severity, when variation was analysed across all 6 treatments in the full model (Eq. 2, Table 3:4). The variation in net blotch severity between leaf layers was different between varieties where as for scald differences between treatments interact with leaf layer and variety.

Variation in the exponential model parameters for both diseases were influenced by the same factors as y_{max} , but some factors only affected variation in one of the two parameters. The estimated initial amount of net blotch was influenced by the treatment and leaf layer, whereas the rate of growth was affected by the variety-leaf layer combination as well as the variety alone. The treatment was very important for both exponential estimated parameters for scald. Variation in these were influenced by this factor in combination with variety (r) and leaf layer (r , y_0) and alone. The variety influenced both parameters but leaf layer alone was only important for the estimated initial amount of disease.

Variation in total disease level under the full model (Eq. 2) was significantly affected by the treatment and variety as well as both the treatment-variety and leaf layer- variety combination.

Maximum total disease level was the only disease parameter analysed in the interaction model (Eq. 3). Treatment was included as the combination of pathogen species applications and only data from treatments 1-4 were included in the analysis (Table 3:5). The variation in total disease level under this model was significantly influenced by application of *D. teres* but not by *R. secalis* application. No significant variation between *D. teres* and *R. secalis* application was found. This means that across the three varieties, the levels of total disease found from the early *D. teres* and *R. secalis* applications alone were additive to the levels found when both were applied together at the same time point. Leaf layer and variety are other major factors affecting the total level of disease, both alone, in combination, and with *D. teres* application. The variation in total disease furthermore is slightly influenced by an interaction between *R. secalis* application and variety ($P=0.07$). The same analysis within each variety showed significant effect of *D. teres* application on all three varieties, with more total disease where this pathogen had been applied, but with a relatively higher increase on Goldie. On this variety was further found an effect of the leaf layer. Total disease level on Punto was affected by more factors than any of the other two. *R. secalis* showed a significant influence on disease variation with leaf layer, with *D. teres* application and alone. The statistical interaction between the pathogens was negative, i.e. total disease levels on Punto were reduced where the two species had been applied together relative to an additive effect.

All three disease measures, scald, net blotch and total disease, were analysed in the time model (Eq. 4). This model compares levels and model parameters from the early pathogen application, under different inoculation situations of the other pathogen. The levels of the factor treatT are defined by the application of the other pathogen, with values non, early or late. Variation in maximum severity (y_{max}) of net blotch was not affected by application scenarios of *R. secalis*, but by leaf layer and variety alone as well as their combination (Table 3:6). Layer was an important determinant of y_0 and so was variety for r . However, for both y_{max} and r , there was a tendency towards reduced levels in treatment 4 (*R. secalis*-early) and treatment 6 (*R. secalis*-late), relative to treatment 3 where no *R. secalis* had been applied (Table 3:3, Figure 3:2).

The maximum scald severity was strongly influenced by treatment, i.e. *D. teres* application, as well as variety and leaf layer (Table 3:6). For the three treatments compared here, the treatment with joint early inoculation of the two pathogens (treatment 4) had the lowest average level of scald and the highest scald severities were found in treatment 5. Variation in estimated initial amount of scald was highly influenced by the different *D. teres* application scenarios and leaf layer were both important for the estimation of y_0 . The combination of treatment and layer, layer alone as well as variety, were important for rate of scald development.

The analyses of y_{max} in the three senescence-subsets produced some results which differed between subsets (data not shown). The differences mainly involved changes in relation to influence of leaf layer, either alone or as interactions with other parameters. There were examples of differences in all three models and the three disease measures.

3.6 Discussion

Pathogen interactions have been shown to influence total disease levels (Madariaga & Scharen, 1986; Nolan *et al.*, 1999) and multiple pests may have significant economical

importance (Blackshaw, 1986). Interactions between *R. secalis* and *D. teres* have previously only been studied in the greenhouse within a single generation (Xue & Burnett, 1995) or in the field at the crop level (Pinnschmidt *et al.*, 2002). Here we investigated the dynamics of net blotch and scald development on single leaves, when these occur in combination over several disease generations in the field. We found significant antagonistic effects between the two pathogens confirming results obtained at high inoculum pressure under controlled greenhouse conditions (Xue & Burnett, 1995).

The difference between varieties in susceptibility towards the pathogens meant that the highest net blotch levels were found on Goldie and the highest scald levels on Punto. Levels for both diseases were intermediate on Brazil. The two diseases developed with different successes; net blotch was present in all plots, whereas scald was practically confined to plots treated with *R. secalis*. The wide distribution of net blotch implied that scald was never observed in the absence of net blotch and conclusions regarding effects of net blotch on scald development are therefore evaluating the influence of different levels of net blotch. Further was net blotch present across all leaf layers whereas scald was mainly confined to lower layers. *R. secalis* might have been limited by climatic conditions being less favourable for scald development. This disease depends on splash dispersal (Skoropad, 1959; Ayesu-Offei & Carter, 1971), whereas *D. teres* is mainly wind dispersed (Shipton *et al.*, 1973). Another possibility is that *D. teres* was introduced with the seed or from stubble in the field, neither of which are likely, based on the early disease check and the fact that barley had not been grown in the field in the previous season.

Differences in disease establishment might be partly due to the different inoculation methods. The early net blotch was introduced via infected straw, which was left in the plots, whereas the late *D. teres* and both *R. secalis* applications were done with spore suspensions, and the straw probably delivered inoculum for a longer period than did the suspension.

The necrotic area constituting disease symptoms does not describe effects of necrotrophic pathogens on the plant alone. The disease also implies increased senescence of the leaf: leaves with higher disease severity have a higher rate of senescence, which has consequences for leaf area dynamics, crop growth rate and yield (Pinnschmidt *et al.*, 1995). In the present study the consequence was, that plants with high levels of net blotch generally had one green leaf layer less than less diseased plants at the later time points (data not shown). Senescent leaf area was here recorded as potentially overlapping with disease symptoms and disease observations represents the total lesion-area on a leaf. However, as symptoms in senescent tissue are probably faded relatively to symptoms in green tissue, disease severity is underestimated at high senescence levels. This underestimation can be problematic if symptoms within the senescent tissue still produce spores and therefore count epidemiologically. On the contrary, if disease assessments were to be made as percentage of green tissue, another problem occurs. When the absolute green area differs between compared crops, severities will be based on different absolute areas and hence comparison of results will be misleading. To be able to discuss this uncertainty of influence of senescent area, analyses of y_{max} were performed for different senescence subsets. Differences in results from the three subsets mainly involved changes in importance of the factor leaf layer. The practical consequence of selecting the three senescence subsets, was that different numbers of data points from different leaf layer samples were included per subset, mainly for the lowest leaf layer, where variation in senescence was highest. The consequence hereof was, that

analysis of variation in disease data related to leaf layers were affected. There is probably not one correct level of senescence under which to analyse the data, but it is an aspect, which must be kept in mind when working with pathogens that might affect the senescence rate.

By preserving leaves it was possible to test the repeatability of the assessments. Assessing disease severity is subjective, and potentially associated with precision errors (Cooke, 1998). A large precision-error implies poor repeatability of the measurement or a high observer-related variation in the data. This error type is important to minimise when comparing development in data over time, to be able to distinguish real development from variation in assessments. A satisfactory precision was obtained in this study.

The single leaf approach to monitoring disease in the field is considerably more time consuming than simply assessing disease at the level of the whole crop. However, it may help to reveal information about pathogen interactions, which are otherwise not observable, by investigating interactions at a low level of detail. The association analysis showed a negative correlation between net blotch and scald on the same leaf, i.e. scald and net blotch are found less severe when occurring together on individual leaves than would be expected if they were independent from each other. Studies of pathogen interactions in controlled environments often investigate interactions by applying pathogens on single leaves and monitoring resulting disease development (Adee *et al.*, 1990; Xue & Burnett, 1995; Al-Naimi *et al.*, 2005). This method does thus not reflect a field situation, where inoculum from different species will infect a leaf at different points in time and under sub-optimal conditions. The association analysis on single leaves is particularly well suited to investigate disease interactions in the field. A precondition for this analysis though, is that both diseases occur at relatively low levels, as a null-hypothesis regarding random distribution is violated at high densities.

The maximum total disease severity (Table 3:4) showed a significant non-additive effect between the two pathogens in the interaction model on Punto, but not on other varieties. This variety was the most susceptible to the scald causing pathogen, *R. secalis*. Given that this disease generally was observed in lower levels than net blotch, it may be that effects between the two pathogenic species are best observed on this variety.

Disease levels caused by each pathogen where applied at the early time point were compared in the time model under the three inoculation scenarios of the other pathogen (Table 3:5, Figure 3:2). In this analysis, no significant influence of *R. secalis* was found for net blotch, though there was a tendency towards reduced net blotch levels when the two pathogens developed simultaneously. This indicates that *R. secalis* can affect *D. teres* development.

Variation in scald levels was significantly affected by the *D. teres* application. The lowest levels were observed in the treatment with early application of both fungi, and the highest when *D. teres* was applied 26 days after *R. secalis*, higher than when *R. secalis* had been applied alone.

Together this indicates, that there is no simple effect of interaction between the two species with respect to relative timing of arrival on the host. Furthermore, the variety may be important for the outcome of the inoculation.

The exponential model fitted data the best, which is in contrast to an intuitive expectation for a system with an obviously limited resource. The logistic model was

found most suitable for describing development of the biotrophic powdery mildew fungus (*Blumeria graminis*) on barley by Østergård & Pons (1996) and Pons-Kühnemann (2005). Beresford & Royle (1991) analysed brown rust (*Puccinia hordei*) on barley by counting number of uredinia per leaf area. The increase in this variable could be described well by an exponential model until the time when leaves became completely senescent. In the present study, the suitability of the exponential model can probably be explained by the close relationship between diseased and senescent leaf area, such that senescence dominated before disease severity reached a level where growth was limited, rendering an exponential disease progress curve. Leaves with more than 50% senescence were discarded in the analysis. Variations in the exponential model parameters (r and y_0) were however to a lesser extent dependent on the factors treatment, variety and leaf layer than the maximum levels of disease (y_{max}). Factors of importance to y_{max} , tended to affect either of the two parameters, i.e. such that the factors associated disease establishment (treatment and leaf layer) influenced variation in y_0 and those associated with the host as a substrate for disease growth (variety and leaf layer) influenced estimates of the growth rate. The treatment factor determines inoculum application, which corresponds to the y_0 estimate and the variety factor describes the suitability of the plant as substrate for disease growth, based on differences in resistance properties. The leaf layers, which had importance for both estimated exponential parameters, both influence disease progress vertically in the canopy, and hence y_0 on individual layers, as well as represent the variety and hence substrate suitability for pathogen development.

The three different approaches (association analysis, y_{max} , and the exponential model estimation) to analysing interactions between simultaneous disease development all produced similar results with respect to interaction effect. The association analysis uses the individual leaf observations to explore effects the physical presence one disease may have on the other. This method produces the most direct analysis of interaction. The y_{max} and regression analysis use averages of the leaf layer samples and summarize the development over time. The results from these two are similar to the extent, that the y_{max} analysis may suffice in most situations.

The three different approaches allow different possibilities for drawing conclusions about density dependence or mechanistic interactions. In the association analysis effects of mechanistic interaction may be distinguished from density dependence, assuming that severity levels of either disease will be distributed randomly across leaves at low severity levels. Thus, results presented here strongly indicate that *R. secalis* and *D. teres* do not develop equally well on a leaf in the presence of each other as they do alone, and hence that some interspecific mechanistic interaction is involved. This conclusion cannot be drawn from analyses of y_{max} or growth model parameters alone though, where averages of observations are analysed and effects of mechanistic interactions and density dependence are confounded. The results thus show, that the single leaf approach can provide information which is valuable in studies on interspecific pathogen interactions.

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3.9 Tables

Table 3:1. Treatments given by application of the two pathogens. Rhynchosporium secalis (scald) was applied by spore suspension on both the early and the late inoculation dates. The early Drechslera teres (net blotch) application was done using infected straw and the late inoculation was done using spore suspension (see text for details). Early = GS 13 (2-4 May); late = GS 25 (28-30 May).

	<i>R. secalis</i> (Rs)	<i>D. teres</i> (Dt)
1	no	no
2	early	no
3	no	early
4	early	early
5	early	late
6	late	early

Table 3:2 Dates for collection of leaf samples in the 3 replications of the trial. Julian date in parentheses.

replication	Date (Julian date)				
1	16/5 (136)	29/5 (149)	12/6 (163)	25/6 (176)	3/7 (184)
2	20/5 (140)	4/6 (155)	17/6 (168)	26/6 (177)	4/7 (185)
3	20/5 (140)	4/6 (161)	19/6 (170)	1/7 (182)	7/7 (188)

Table 3:3 Average maximum disease severity, y_{max} , of net blotch, scald and total disease across epidemic replicates. For treatments see Table 3:1

Variety		Brazil			Goldie			Punto		
Disease		Nbt ^a	Sct ^b	Tot ^c	Nbt	Sct	Tot	Nbt	Sct	Tot
Treatment	Leaf layer									
1	F-1	0.68	0.00	3.25	2.03	0.61	4.03	0.79	0.04	3.14
	F-2	0.58	0.00	2.68	4.09	0.07	4.59	1.14	0.10	2.69
	F-3	0.67	0.29	2.19	2.28	0.09	3.14	1.05	0.24	1.71
2	F-1	0.29	2.25	4.70	2.34	0.05	2.62	0.09	1.62	3.62
	F-2	0.40	0.96	3.00	2.37	0.29	3.39	0.68	2.08	4.12
	F-3	0.22	3.64	4.06	1.39	1.94	3.17	0.24	4.36	4.64
3	F-1	4.02	0.00	4.48	11.22	0.00	11.22	2.91	0.08	4.52
	F-2	5.90	0.16	6.06	17.44	0.06	17.65	3.83	0.05	4.38
	F-3	3.27	1.37	5.04	6.94	0.18	7.19	3.69	0.62	4.78
4	F-1	3.77	0.48	4.41	11.41	0.22	11.63	2.85	0.38	3.90
	F-2	4.69	0.81	5.13	17.02	0.00	17.02	4.42	0.57	5.02
	F-3	2.85	1.87	5.58	6.52	0.56	7.19	3.36	2.47	5.61
5	F-1	0.44	0.31	3.81	3.51	2.00	6.06	0.29	4.04	4.85
	F-2	1.12	0.89	3.17	3.14	0.59	5.31	0.47	1.75	3.74
	F-3	0.69	4.76	5.28	1.42	1.79	3.29	0.35	4.52	4.75
6	F-1	3.39	0.24	5.31	11.28	0.08	11.28	2.79	0.29	3.37
	F-2	4.16	0.98	5.59	10.72	0.76	10.76	4.67	2.18	6.29
	F-3	4.63	1.21	6.87	7.29	0.55	7.46	4.94	1.52	5.93

a: Maximum disease severity of net blotch

b: Maximum disease severity of scald

c: Maximum disease severity of total disease

Table 3:4 Analysis of variance of y_{max} for net blotch, scald and total disease; and parameters from the exponential model for net blotch and scald. Analyses were made for data from all 6 treatments (treatA). Shown are P -values. Number of observations included in analyses are given below each column

Disease	Nb ^a			Sc ^b			Tot ^c
Variable	Y_{max}	y_0	r	Y_{max}	y_0	r	Y_{max}
Factor							
treatA	<0.001	<0.001	0.81	<0.001	<0.001	<0.01	<0.001
Layer	<0.001	0.04	0.19	<0.001	<0.001	0.13	0.12
Variety	<0.001	0.14	<0.001	<0.001	0.03	0.03	<0.001
treatA :Layer	0.67	0.07	0.48	0.02	<0.001	<0.001	0.29
treatA :Variety	0.58	0.87	0.07	<0.01	0.33	0.04	<0.001
Layer:Variety	0.03	0.95	<0.01	0.14	0.08	0.08	<0.001
treatA:Layer:Variety	0.90	0.87	0.75	0.83	0.69	0.62	0.67
	162	152	152	162	155	155	162

a: Maximum disease severity of net blotch

b: Maximum disease severity of scald

c: Maximum total disease severity

Table 3:5 Analysis of variance of y_{max} for total disease. The model tests additive effects of *D. teres* and *R. secalis* applications on total disease level. This model includes only data from treatments 1 to 4 (Table 3:1). In the table are shown P-values. Number of observations included in analyses are given below the column.

Variety	Across varieties	Brazil	Goldie	Punto
Factor				
Dt	<0.001	<0.001	<0.001	<0.001
Rs	0.23	0.33	0.42	<0.001
Layer	0.05	0.99	0.01	0.66
Variety	<0.001			
Dt :Rs	0.24	0.22	0.51	<0.01
Dt:Layer	0.18	0.30	0.09	0.06
Rs:Layer	0.12	0.46	0.81	0.01
Dt:Variety	<0.001			
Rs:Variety	0.07			
Layer:Variety	0.02			
Dt:Rs:Layer	0.50	0.86	0.87	0.21
Dt:Rs:Variety	0.15			
Dt:Layer:Variety	0.05			
Rs:Layer:Variety	0.90			
Dt:Rs:Layer:Variety	1.00			
	108	36	36	36
a: Maximum total disease severity				

Table 3:6 Analysis of variance of ymax for net blotch and scald and parameters from the exponential model for the two diseases across variety due to influence of a subset of treatments (treatT), leaf layer (layer) and variety (var). In the table are shown P-values Number of observations included in analyses are given below each column

Disease	Nb ^a			Sc ^b		
Variable	Y_{max}	y_0	r	Y_{max}	y_0	r
Factor						
TreatT ^c	0.91	0.29	0.54	<0.001	<0.001	0.80
Layer	<0.001	0.05	0.51	<0.001	<0.001	0.06
Variety	<0.001	0.23	0.01	<0.001	0.07	0.05
TreatT:Layer	0.26	0.19	0.29	0.79	<0.001	0.02
TreatT:Variety	0.67	0.83	0.75	0.06	0.53	0.17
Layer:Variety	<0.01	0.98	0.30	0.39	0.28	0.18
TreatT:Layer:Variety	0.98	0.59	0.86	0.29	0.71	0.69
	81	76	76	81	76	76

a: Maximum disease severity of net blotch

b: Maximum disease severity of scald

c: For net blotch, treatT is treatment 3,4 or 6 (i.e. D. teres = early and R. secalis = no, early and late respectively).

For scald, treatT is treatment 2,4 or 5 (i.e. R. secalis=early and D. teres = no, early and late respectively).

3.10 Figures

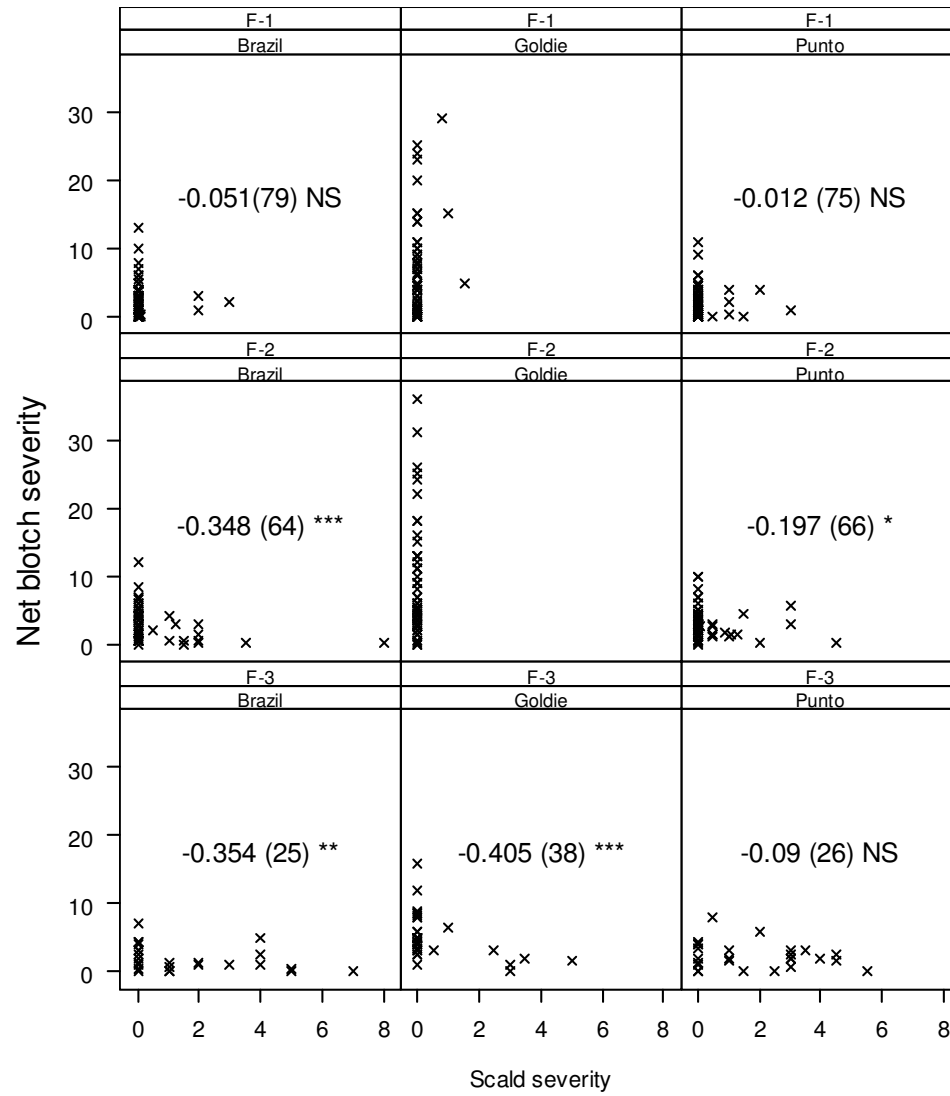


Figure 3:1 Associations between observations of net blotch and scald on individual leaves, for three leaf layers and three varieties. Data are from the last three sampling dates of treatment 4. The value for Kendall's τ and number of data points in the analysis (N) are shown. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

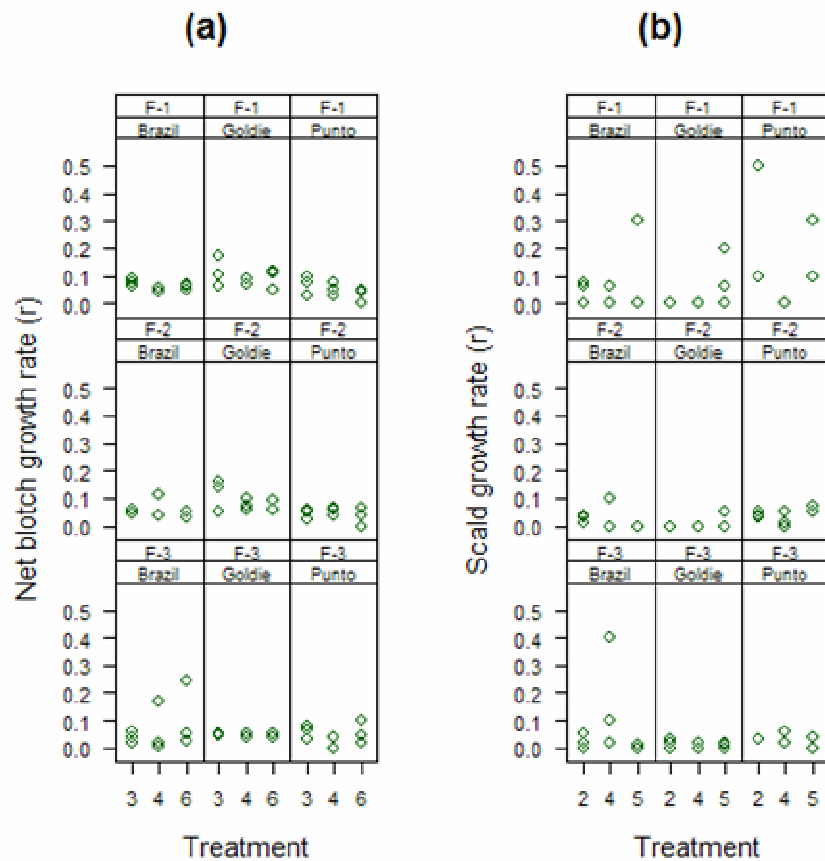


Figure 3:2 Estimated growth rates (r) from the exponential model for three varieties, three leaf layers and three replicates for net blotch (a) and scald (b). The three treatments for either disease correspond to application of the causing pathogen at the early time point and three different application scenarios of the other pathogen: non, early or late corresponding to treatment 3,4 or 6 and 2,4 or 5, respectively, for net blotch and scald.

4 Simulation of effect of leaf layer dynamics and vertical spore dispersal on competition between foliar pathogens

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4.1 Abstract

Diseases developing on a crop are not faced with a constant leaf area surface to develop over, but rather discrete areas in space and time, decided by development of single leaves and leaf layers of the plant. Density dependence is important to consider when evaluating foliar disease progress, due to the dependence on leaf area for development. Often, more than one disease develops in a field at the time. This has lead to research exploring potential antagonistic or synergistic effects between the two species. A logistic model for two simultaneously developing pathogen species, considering no antagonistic or synergistic effects but only density dependence has illustrated that in a two species situation, expected total disease level of the two pathogens is not a simple a sum of levels in the former. This is here expanded, to focus on the two pathogens and the effect of leaf layer development for observed final levels of the two diseases. A model is used to evaluate the effect of density dependence, leaf layer dynamics, differences in pathogen epidemic parameters and vertical spore dispersal, on simultaneous development of two foliar pathogens *Rhynchosporium secalis* and *Drechslera teres*. Epidemic parameters for the two species would indicate that *R. secalis* has a higher growth rate than *D. teres*, and would therefore be expected to be the dominant disease, if progress is simulated without consideration of canopy structure. The simulations showed that dispersal parameters vertically in the canopy influenced the dynamics, such that when leaf layer dynamics were considered, the dominating species was *D. teres*. The model has illustrated, that the combination of density dependence and leaf layer dynamics influence the outcome of development of two diseases in a field, such that it is impossible simply from observations on disease levels to draw conclusions regarding interactions between pathogens, as expected levels in the two-species situation can not be described from the single-species observations.

4.2 Keywords

Pathogen interaction, simulation model, ModelMaker

4.3 Introduction

Foliar pathogens developing over several generations in a crop are not faced with a constant leaf area resource for disease development, but rather individual leaves, which are present as discrete entities in space and time. This means pathogens are dependent on their ability to follow crop dynamics to maintain themselves. The crop dynamic importance has been explored for single pathogen species (Madden, 1997; Lovell *et al.*, 1997; Pielaat *et al.*, 2002; Lovell *et al.*, 2004).

An important aspect related to foliar disease development and the nature of their resource, the leaf area, is density dependence, the effect on population growth from a decreased rate of growth due to increased population density (Begon *et al.*, 1996). Density dependence is fundamental for foliar disease progress, due to a number of characteristics of the pathogen-plant relationship. 1) The pathogen depends on the leaf surface for sporulation. 2) The leaf area is finite, *i.e.* numbers cannot overshoot a sustainable maximum size 3) Area infected, can generally not become de-infected, *i.e.* become available for others to infect (at least for necrotrophic pathogens).

In reality though, disease is rarely given by only a single pathogen species in a given crop (Kranz & Jörg , 1989), which has promoted interest in the potential interactions between multiple pathogens, generally aiming to draw conclusions on antagonistic or synergistic effects between pathogens. A simple logistic model has been used to illustrate the role of density dependence on disease levels of two simultaneously developing foliar pathogens (Vollmer *et al.*, 2005a), and this illustrated that it is no trivial task to draw conclusions regarding competition between two co-developing foliar diseases with respect to distinguishing the two types of competition: exploitation (density dependence) and interference (antagonism).

To analyze multiple pathogen developments in a crop, it is important to realise how the crop or leaf layer dynamics and density dependence influence disease levels and how these might affect conclusions regarding interactions. In this paper, the objective is to explore further the effect of canopy dynamics and density dependence on simultaneous development of two pathogens over emerging and senescing leaf layers, by only considering these two elements and not any other types of interaction. From this is discussed the problems associated with analysing simultaneously developing disease epidemics, when the aim is to draw conclusions regarding synergistic and particularly antagonistic effects.

4.4 Model and Parameters

The model is developed for the pathosystem spring barley (*Hordeum vulgare*), *Rhynchosporium secalis* (causes scald), and *Drechslera teres* (causes net blotch). These two pathogens are ideal examples for exploring importance of density dependence between two simultaneously developing species, as both are necrotrophic and develop well described areas of lesions, which do not overlap on leaves.

The plant area is described with separate leaf layers that emerge and senesces continuously over the simulation period. Epidemic developments of the two fungi are described coupled with their spring barley host over a cropping season, *i.e.* from emergence of the first leaf until the last has been removed.

The terms pathogen and disease are used almost synonymously throughout the paper, such that the unit for pathogen quantification is area of leaf, which is assumed equivalent to diseased area. This ignores any potential effect of virtual lesion size (Bastiaans, 1991), or the effect that only a proportion of the pathogen infected leaf area may be represented as visible disease symptoms. This is likely to be important for actual pathogen development, but will not affect conclusions drawn from the present model.

4.4.1 Leaf area development

Number of leaves developed by a barley plant may differ between varieties. Eight leaf layers are modelled here, which is the number observed on the spring barley varieties Goldie and Punto, ignoring the flag leaf, which comprises an insignificant area on barley

(Figure 4:1). Furthermore, areas of leaves of different layers have different sizes (Figure 4:1), which is simplified here, to include only two size-categories or leaf groups (fl_k) (Table 4:3). $LM_{l,t}$ is the area of a given leaf layer (l) at time t . The full area of a leaf layer emerges on the layer specific date of emergence (ed_l). This implies that the model only describes leaf dynamics of a single main tiller, as the value for fl_k corresponds to the area of a single leaf. Values for emergence dates and average leaf duration for the 8 leaf layers are taken from unpublished field observations of spring barley (Figure 4:1). State variables are shown in Table 4:1, indices in Table 4:2 and parameters used to describe leaf area development are shown in Table 4:3.

Even in the absence of disease, leaves do not remain green, but are affected by natural senescence. The dynamics of the area of a leaf layer ($LM_{l,t}$) is thus modelled, by a period with full leaf size, the length of which is determined by a constant leaf layer duration (ld) in the absence of disease. When the senescence process is initiated, the area is reduced linearly, determined by duration of the senescence period (sn), which is constant. Some pathogens may have an accelerating effect on leaf decay (Savary & Zadoks, 1992; Pinnschmidt *et al.*, 1995; Vollmer *et al.*, 2005b), which could affect leaf dynamics, and a senescence inducing constant (si) is therefore included, which together with the total area covered by lesions on the leaf layer ($Y_{d,l} + Y_{r,l}$) determines the actual duration until onset of senescence. Equations 1-3 describe leaf area development.

$$\begin{aligned} \text{If : } t < ed_l \\ LM_{l,t} &= 0 \end{aligned} \quad (1)$$

$$\begin{aligned} \text{If : } ed_l < t < ed_l + ld \times \left(1 - si \times \frac{Y_{d,l} + Y_{r,l}}{fl_k} \right) \\ LM_{l,t} &= fl_k \end{aligned} \quad (2)$$

$$\begin{aligned} \text{If : } t > ed_l + ld \times \left(1 - si \times \frac{Y_{d,l} + Y_{r,l}}{fl_k} \right) \\ LM_{l,t} = \max \left(0; \frac{fl_k}{sn} \times \left[sn + ld \times \left(1 - si \times \frac{Y_{d,l} + Y_{r,l}}{fl_k} \right) + ed_l - t \right] \right) \end{aligned} \quad (3)$$

4.4.2 Disease growth

To simulate development of *R. secalis* and *D. teres*, a set of parameters have been chosen, which are considered general for the two species under ideal environmental conditions regarding temperature, rainfall and humidity and a host variety with similar susceptibility to both pathogens.

Growth of the pathogens is described via the three epidemiological stages of a lesion, latent ($L_{i,l}$), sporulating ($S_{i,l}$) and post-sporulating ($P_{i,l}$) area, by a compartmental model (Segarra *et al.*, 2001) with these as respective state variables for species i (Table 4:1). Total diseased area on a leaf layer for species i at time t ($Y_{i,l,t}$) is the sum of the three types of lesioned area ($L_{i,l,t} + S_{i,l,t} + P_{i,l,t}$).

The species-specific latent period (lp_i) and sporulating period (sp_i) determines the rates of transfer between categories from the latent to sporulating area and sporulating to post-

sporulating area respectively (Table 4:4). The latent period is set at 14 days for *R. secalis* (Jackson, 1997) and 10 days for *D. teres* (Shaw, 1986). Skoropad (1966) found that a scald lesion ceased to sporulate after 16 days at 18°C, which forms the basis of duration of sporulation period for this species. No estimate of infectious period has been found for *D. teres* and the same value is therefore used. Establishment of new lesions is a complex function of sporulation rate, successful spore dispersal (*i.e.* removal by wind or water from conidiophore, transport and landing on susceptible leaf area), spore germination and infection. The parameters describing this process are mostly highly environmentally dependent and difficult to quantify, and quantification generally have been made under controlled environmental conditions. In the present model this part of the epidemic is therefore summarised in the species specific parameter termed reproductive potential (rp_i), which quantifies the total number of potential new lesions produced over time per sporulating area unit (Table 4:4).

The values are set to reflect relative differences in the spores produced per area of the two species and have with this in mind been adjusted such that the diseases reach similar levels after 90 days when occurring alone.

About 4×10^4 spores from *D. teres* have been observed from an inoculated plant at GS 13, which can be considered to represent the maximum possible spore production (Shaw, 1986). Spore production by *R. secalis* has been observed as high as 5×10^5 spores from a single lesion (Ayesu-Offei & Carter, 1971) and this is assumed to originate from one original spore. From this is taken, that sporulation potential in *R. secalis* is considerably higher than in *D. teres*, but how this relation is with respect to infection and germination is unknown, though it would be expected that rates here were higher for *D. teres* based on larger spores. Since the model focuses on differences in values rather than it applies accurate values, it does not seem unreasonable to use a value for rp_r which is 50% higher than the one for *D. teres* (rp_d) (Table 4:4).

Lesion growth can be a significant component in epidemiology of some diseases (Lannou *et al.*, 1994; Berger *et al.*, 1997). In this model the latent area also increases from lesion growth, determined by the lesion growth rate (lg_i), estimated at 0.20 cm day⁻¹ for *R. secalis* (Xue & Hall, 1991) and 0.16 cm day⁻¹ for *D. teres* (Shaw, 1986) (Table 4:4).

The model accounts for dispersal of spores between leaf layers, such that these may disperse one leaf layer up or down in the canopy. For both pathogens it is assumed that 50% of the spores produced on a leaf layer remain and infect the same leaf layer. The species specific parameter du_i determines the proportion of the remaining spores dispersing one layer upwards in the canopy and the rest (dd_i) disperse downwards. Values reflect the dominant dispersal mechanism of the two species, wind dispersal for *D. teres* (Shipton *et al.*, 1973) and splash dispersal for *R. secalis* (Skoropad, 1959; Ayesu-Offei & Carter, 1971). Vertical spore dispersal ability of *R. secalis* was measured by Fitt (Fitt *et al.*, 1988), who observed that ca 25 % of spores under simulated rain conditions were splashed beyond 10 cm, which is the approximated distance observed on the varieties from where leaf development data are taken (data not shown). Given that this was under conditions of homogeneous rainfall, and in all directions, a lower value is used in the model, with du_r set to 0.15. No data on vertical dispersal of *D.teres* have been found, but is assumed to be somewhat higher, and du_d is therefore set at 35 % (Table 4:4).

The full model description is given in equations 4-6. The rate of growth, *i.e.* the proportion of the reproductive potential which is allowed to infect non-diseased and non-senescent leaf area, is regulated by the green leaf area available for infection ($GL_{l,t} = LM_{l,t} - Y_{r,l,t} - Y_{d,l,t}$). In the following differential equations the t 's are omitted

If : $GL_i > 0$

$$\begin{aligned}\frac{dL_{i,l}}{dt} &= \left[1 - \frac{Y_{d,l} + Y_{r,l}}{LM_i} \right] \left[\frac{rp_i}{sp_i} \times (du_i \times S_{i,(l-1)} + 0.50 \times S_{i,l} + dd_i \times S_{i,(l+1)}) + lg_i \times L_{i,l} \right] - \frac{L_{i,l}}{lp_i} \\ \frac{dS_{i,l}}{dt} &= \frac{L_{i,l}}{lp_i} - \frac{S_{i,l}}{sp_i} \\ \frac{dP_{i,l}}{dt} &= \frac{S_{i,l}}{sp_i}\end{aligned}\quad (4)$$

If : $GL_i < 0; L_{i,l} > 0$

$$\begin{aligned}\frac{dL_{i,l}}{dt} &= -\frac{L_{i,l}}{lp_i} \\ \frac{dS_{i,l}}{dt} &= \frac{L_{i,l}}{lp_i} - \frac{S_{i,l}}{sp_i} \\ \frac{dP_{i,l}}{dt} &= \frac{S_{i,l}}{sp_i}\end{aligned}\quad (5)$$

Else :

$$\begin{aligned}\frac{dL_{i,l}}{dt} &= 0 \\ \frac{dS_{i,l}}{dt} &= \frac{L_{i,l}}{lp_i} - \frac{S_{i,l}}{sp_i} \\ \frac{dP_{i,l}}{dt} &= \frac{S_{i,l}}{sp_i}\end{aligned}\quad (6)$$

Differences in epidemiological parameters between the two species are found in the latent period (lp_i), reproductive potential (rp_i) and lesion growth rates (lg_i) (Table 4:4). *D. teres* has a shorter latent period than *R. secalis*, and the latter has a higher spore production than the former. Other than this, the two pathogens have different dispersal rates (dd_i and du_i).

Simulation is started by an initial amount of 0.1 cm² latent area for both diseases on the lowest leaf layer. The time unit for simulation time is days, and the model is simulated for 90 days, reflecting the duration of the growth of a spring crop in Denmark.

4.4.3 Evaluation of competition

To evaluate the relationship between the two species, the ratio between them is used. Converting the amount of disease from the two species into a single measure of competition is preferable for presentation of sensitivity analyses and is independent of the total amounts. The total amount of each disease per plant is calculated as the sum across leaf layers.

$$rd_t = \frac{Y_{d,t}}{Y_{d,t} + Y_{r,t}} \quad (7)$$

4.4.4 Software and simulation

The models was simulated using Model Maker version 3.0.3 (Walker & Crowder , 1997) using the Runge-Kutta 4th order integration algorithm .

4.5 Results

A range of scenarios was simulated by varying pathogen dispersal parameters, leaf layer duration and the senescence inducing parameter, or the impact of disease levels on leaf senescence.

The default development of leaf area for the leaf layers, *i.e.* in the absence of disease, is shown in Figure 4:2. It is evident that the maximum total leaf area is 72 cm², as the model considers one plant as representative for the crop.

To investigate the importance of difference in epidemic parameters for the two diseases, disease developments have been simulated to reflect situations where diseases develop either alone or together, with similar and different dispersal rates between leaf layers (Figure 4:3). Using the same dispersal rate, with a quarter of the reproductive spores dispersing one layer up or down respectively ($du_i = dd_i = 0.25$) (Figure 4:3 A, B), *R. secalis* reaches the highest disease level when occurring alone, and when the two are together it exploits almost the complete leaf area severely affecting levels of *D. teres*, due to a higher growth rate from rp_r and lesion growth rate (lg_r). Using the default parameter values for the dispersal parameters (Table 4:4) the two diseases reach similar levels of disease when occurring alone (Figure 4:3 C) and when they occur together *D. teres* reaches a higher level than *R. secalis* (Figure 4:3 D).

Variation in duration, or life length, of a leaf layer, was varied between 10 and 50 days and consequent leaf areas occupied by the two pathogens are shown in Figure 4:4. Sensitivity to variation in leaf layer duration is highest for *D. teres*, but for both diseases a reduction in leaf layer duration has a proportionally larger effect than an increase. The effect of variation in leaf layer duration on the relationship between the two, or the proportion of total disease level comprised by *D. teres* is shown in Figure 4:5, where for all considered values of ld , *D. teres* constitutes the highest proportion after 90 days, *i.e.* larger than 0.5. This relation is variable with time though, and earlier in the time period *R. secalis* dominates.

The role of dispersal abilities on the relative levels of the two diseases was explored by sensitivity analyses where du_i was varied between 0.05 and 0.50 for both species. The result for the proportion of *D. teres* of total disease is shown in Figure 4:6. Dispersal rates for *D. teres* are required to be relatively higher than those of *R. secalis* to dominate in disease level, and consequently *R. secalis* is dominant over a wider range of dispersal values than *D. teres*. Performing a sensitivity analysis with the default values for dispersal for one species and varying this parameter for the other species between 0.05 and 0.5 explored the effect on disease levels. The resulting areas of the two pathogens are shown in Figure 4:7. Changing the default value for du_r of 0.15 has a larger influence than changing the parameter for *D. teres*.

The impact of disease acceleration of senescence was explored by a sensitivity analyses with values of si varying between 0 and 0.50. Figure 4:8 shows that this has more impact

on *D.teres* than *R.secalis*. The change in *si* has little influence on *rd* though, and *D.teres* remains the dominant species across all applied values (data not shown).

4.6 Discussion

Plant diseases in the field rarely occur as single independent events, but most often several diseases are present and develop simultaneously. To be able to understand epidemiology of single species, it is important to establish if antagonistic or synergistic interactions influence co-occurring species, which requires adequate analyses of disease data from the field. For this proper hypotheses must be formulated, and it has been illustrated that simply due to density dependence (exploitation competition) it is no trivial task to draw conclusions on antagonistic effects, as the two types of interaction are difficult to distinguish based on disease data recorded in proportion to the crop alone (Vollmer *et al.*, 2005a). This aspect was further explored here, to consider how different properties of the crop influence competitive outcome between simultaneously occurring pathogens, when only density dependence is considered as interaction type.

The model illustrated that dynamics of the canopy, the leaf layer duration, as well as differences in vertical dispersal rates can have significant impact on competitive outcome from two simultaneously developing pathogens when influenced by only exploitation competition (Figure 4:4). Impact of density dependence is evidenced by comparing levels of individual diseases between the single (A, C) against the corresponding two-species situations (B, D). In both cases, the two pathogens reach lower levels when they are together relative to the single-species situations, and the differences are not given by similar proportional reductions in neither of the cases. The situation with similar dispersal values (A, B) corresponds to the simple two species logistic model (Vollmer *et al.*, 2005a) where the species with highest growth rate alone 'wins' when the two are together. Performing the simulations with the default dispersal values (Table 4:1) the competitive outcome changes, thus illustrating the potential influence of crop properties on co-occurring pathogens.

The effect of relation between leaf layers and dispersal on pathogen dynamics has been considered by Pielat *et al.* (2002) who found that for the splash dispersed *Pyrenopeziza brassicae* on oilseed rape (*Brassica napus*), in the absence of rain the plant was able to outgrow the pathogen, due to abscission of diseased leaves before inoculum had been transferred to higher leaf layers. Thus a too low dispersal rate had serious consequences for the epidemic development. Here we found that accumulated area occupied by both pathogens decreased with decreasing *ld* values in about similar proportions (Figure 4:4). The lower disease levels with shortened leaf life span can be explained by less time for pathogens to develop on a layer, both from new lesion establishments and lesion growth. The length of *ld* did not have dramatic effect on the relation between the two species (Figure 4:5). Early in the simulated period *R. secalis* was dominant, due to higher within leaf layer growth rates whereas later *D.teres* became dominant, from an increasing advantage in vertical dispersal with appearance of new leaf layers. For lower *ld* values though, the competitive advantage was higher than at higher values. Thus, the simulation also illustrated the impact on disease levels of differences in leaf area availability relative to the dispersal rate, which may have epidemic consequences, but it did not affect the competitive outcome.

The sensitivity to senescence acceleration from disease levels was no different between the two diseases, and did not change the relation between levels of the pathogens either. The disease inducing senescence has been observed to affect pathogen interactions though.

A simulation model for multiple pest species in rice coupled to a whole-crop model showed that the acceleration of senescence caused by disease was the most important damage factor for biomass (Pinnschmidt *et al.*, 1995). This may thus be very important for crop performance and consequently disease levels, which corresponds the situation on Figure 4:8, where accumulated areas covered by the pathogens decrease with increasing values of *si*. With increasing senescence the area covered by disease may reach lower absolute values, but crop performance is affected by senescence instead. This influences inoculum production and a high *si* value may cause a reduction in total disease levels (van den Berg & van den Bosch, 2004). With respect to pathogen interactions, observations of two diseases with different impact on senescence acceleration and leaf layers were made by both Savary & Zadoks (1992) and Chester (1944). Savary & Zadoks observed that *Cercosporidium personatum* on groundnut (*Arachis hypogaea*) caused defoliation, which influenced development of *Puccinia arachidis*. Chester observed *Puccinia triticina* and *Septoria nodorum* development in the field and found that the latter caused a devastation of the leaves which practically removed host substrate from the former. This emphasizes the importance of considering different senescence accelerating impact in future simulations.

The present model is based on a range of simplifications, relative to natural disease development. Environmental factors play a crucial role for epidemic development: temperature and humidity along with resistance properties and nutritional status of the host jointly play a major role in determining epidemic potential from the primary inoculum. Here conditions were assumed equally favourable throughout the simulation period. In the field, environment often provides alternating favourable conditions to two competitors, affecting the inter-specific dynamics by giving temporary advantage to one species over the other, acting to influence the relationship decided by duration of this and the basic parameters.

The simulated dynamics are relevant for several other combinations of foliar pathogens exploiting leaf area. The examples used here were *R. secalis* and *D. teres*, but the results are equally relevant to other pathogen-combinations, where it may be assumed that diseases caused by the two species do not overlap on leaf area and that disease can not be removed, *i.e.* infected leaf area does can not become available for either species to infect again.

It has here been illustrated that outcome from simultaneous development of two pathogens even in the absence of antagonistic or synergistic effects is a complex product of lesion growth rates, spore production, dispersal and leaf layer durations. This emphasizes the importance of considering the discrete nature of the host substrate when the objective is to understand interaction between pathogens developing in a crop. Here only the vertical dynamics were considered, but future approaches should also consider how the discrete nature of individual leaves within leaf layers, might influence interspecific dynamics, whether including only exploitation competition or also interference competition.

4.7 References

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4.8 Tables

Table 4:1 State variables used in the model

State variable	Description	Unit
Leaf Area		
$LM_{l,t}$	Max area of leaf layer l for infection at time t	cm^2
$GL_{l,t}$	Green leaf area of leaf layer l at time t $= LM_{l,t} - Y_{r,l,t} - Y_{d,l,t}$	cm^2
Disease		
$L_{i,l,t}$	Latent tissue for species i on layer l at time t	cm^2
$S_{i,l,t}$	Sporulating area for species i on layer l at time t	cm^2
$P_{i,l,t}$	Postsporulating tissue for species i on layer l at time t	cm^2
$Y_{i,l,t}$	Area infected tissue for species i on layer l at time t $= L_{i,l,t} + S_{i,l,t} + P_{i,l,t}$	cm^2
$Y_{to,l,t}$	Area of total infected tissue on layer l at time t $= Y_{r,l,t} + Y_{d,l,t}$	cm^2
rd_t	Proportion of disease caused by <i>D. teres</i> of total disease level at time t .	-

Table 4:2 Indices used in the model

Index	Description	values
t	Time	0:90
i	Species	d: <i>D. teres</i> , r: <i>R. secalis</i> to: Total disease
l	Leaf layer	1:8
k	Leaf layer group	up: Upper 4 layers lo: Lower 4 layers

Table 4:3 Parameters used to describe leaf area simulation in the model

Parameter	Description	Unit	Values
fl_{up}	Full leaf size, for the upper 4 leaves	cm ²	6
fl_{lo}	Full leaf size, for the lower 4 leaves	cm ²	12
ed_l	Leaf layer emergence date for layer l	day	$ed_l = 7 * (l-1)$
ld	Leaf layer duration in the absence of disease	days	25
sn	Duration of senescence period	days	10
si	Disease increasing senescence	-	0.2

Table 4:4 Parameters used for simulation of disease

Parameter	Description	Unit	<i>R. secalis</i>	<i>D. teres</i>
lp_i	Latent period for species i	days	14	10
sp_i	Sporulating period for species i	days	16	16
rp_i	Lifetime reproductive potential per cm ² for a lesion of species i	Number of infecting spores/cm ² /infectious period	15	10
lg_i	Lesion growth rate for species i	cm ² /day	0.20	0.16
du_i	Proportion of spores dispersing upwards in canopy for species i		0.15	0.35
dd_i	Proportion of spores dispersing downwards in canopy for species i = 1-0.5- du_i		0.35	0.15

4.9 Figures

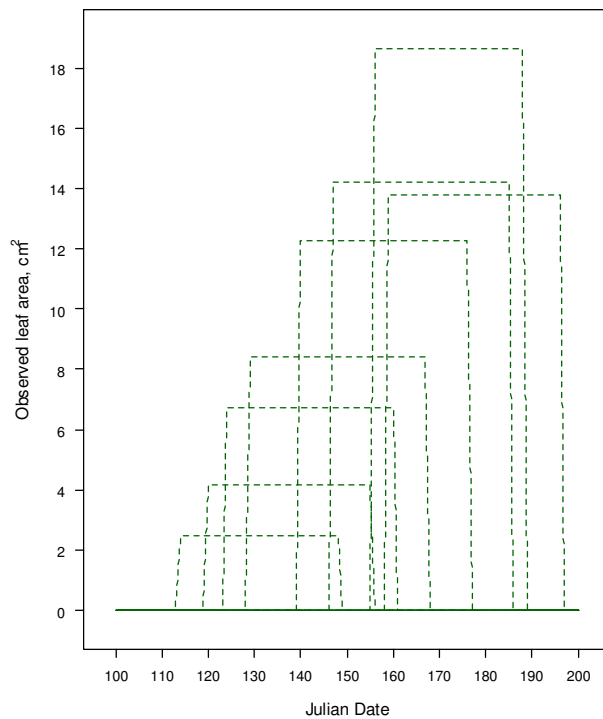


Figure 4:1 Observed leaf layer developments of the spring barley variety Goldie from a field trial in 2003, in a plot with minimal levels of disease. Each curve represents a leaf, with the leftmost being the first and the rightmost the last, or the leaf below the flag leaf. The flag is not included.

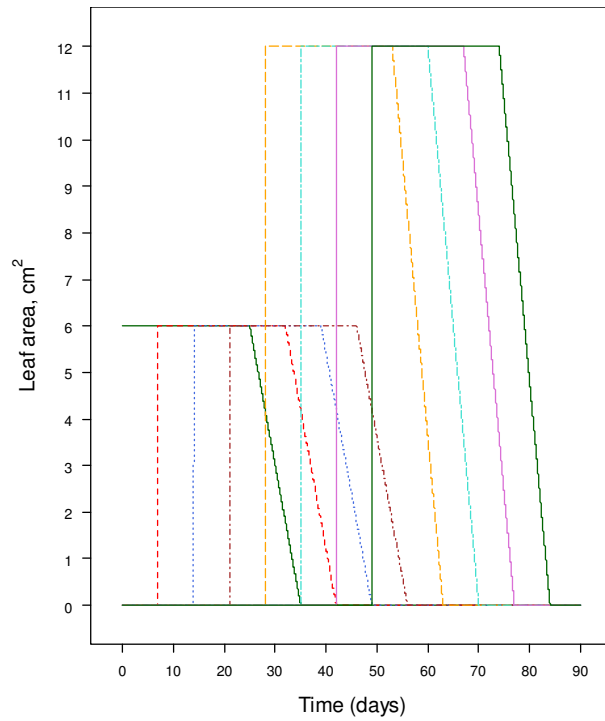


Figure 4:2 Leaf area developments for 8 leaf layers in the absence of disease induced senescence, i.e. $si=0$. The four lower leaves have a maximum size of 6 cm^2 and the upper four a maximum size of 12 cm^2 .

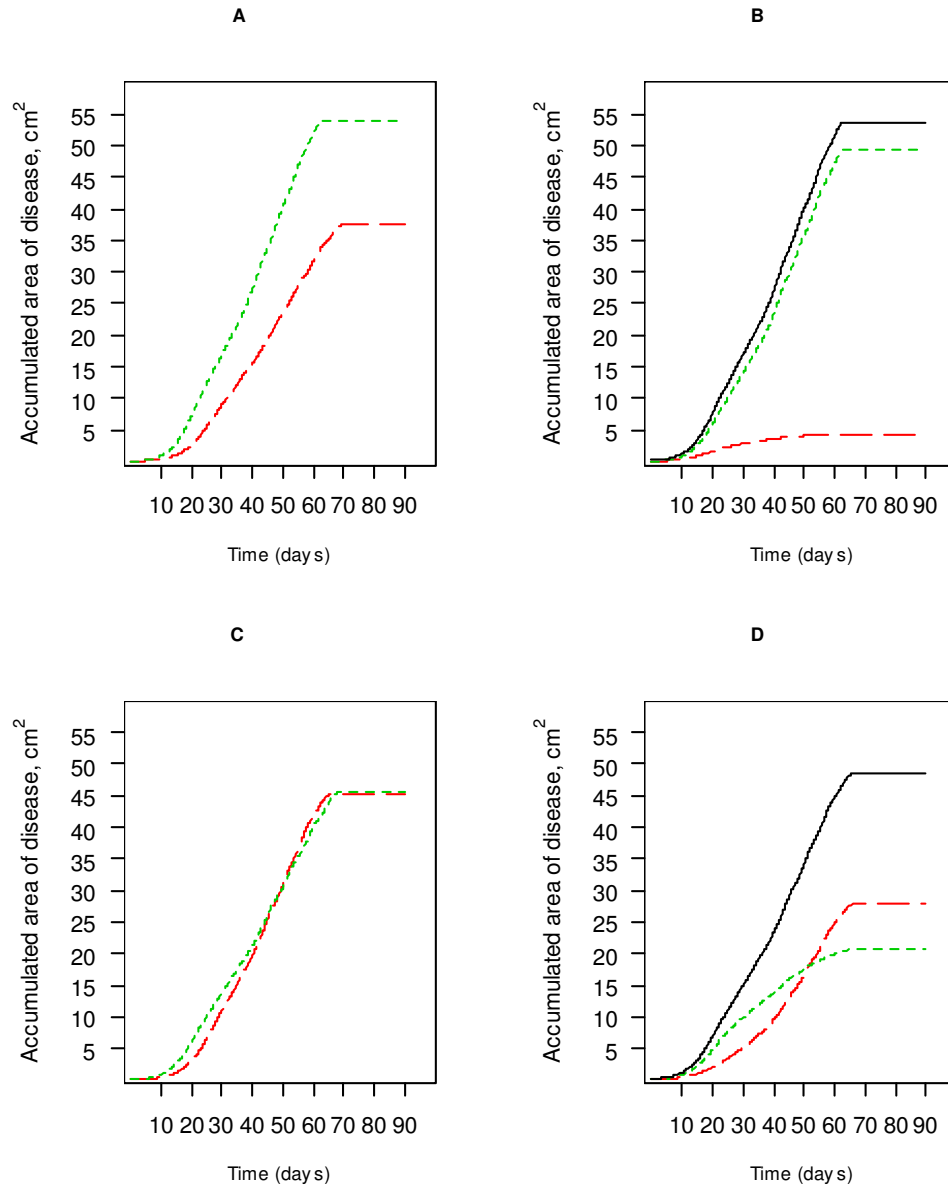


Figure 4:3 Development in *D. teres* (wide broken line) and *R. secalis* (dashed line) and total disease (full line) when developing separately (A, C) and together (B, D), either with the same dispersal rates between leaf layers, $du_{R. secalis} = du_{D. teres} = 0.25$ (A, B) or with different rates, $du_{R. secalis} = 0.15$ and $du_{D. teres} = 0.35$ (C, D)

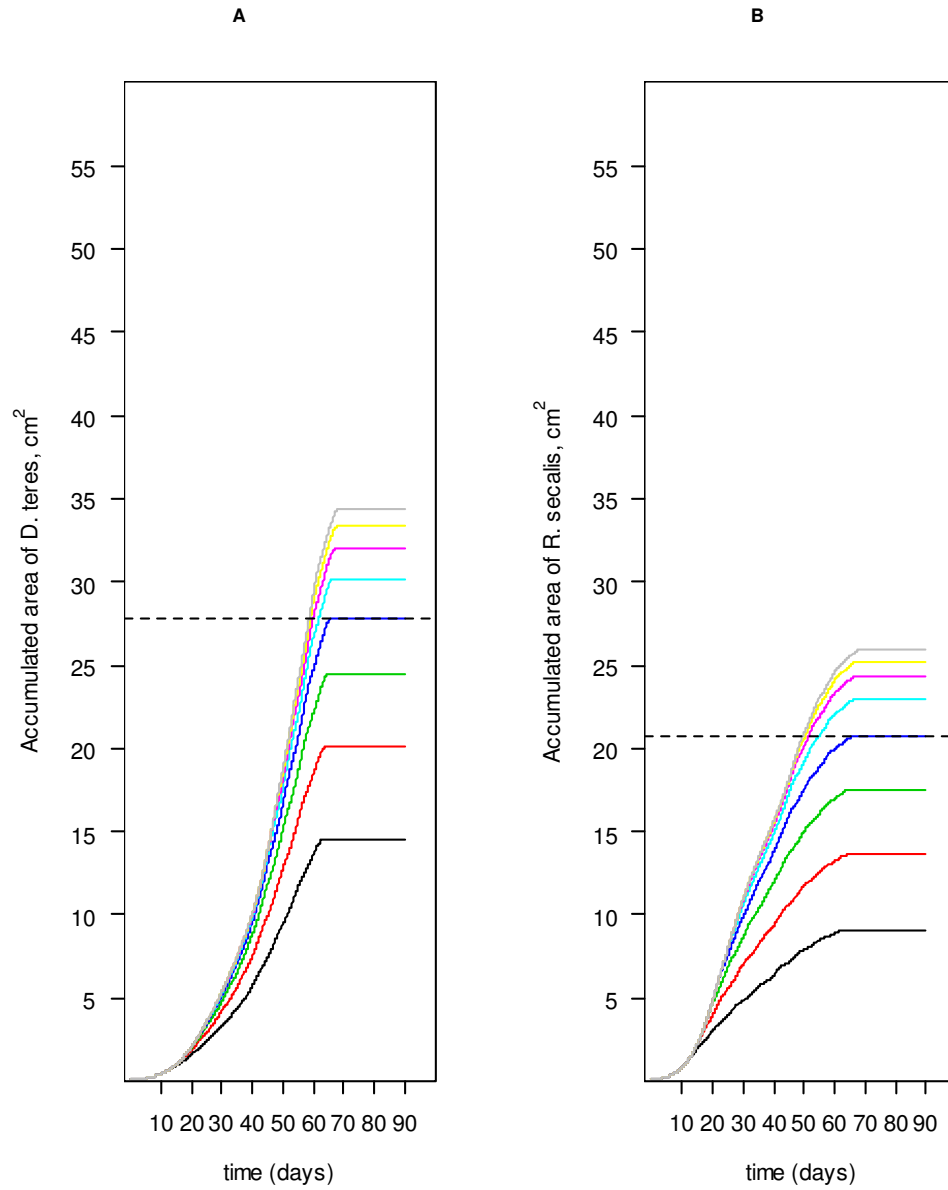


Figure 4:4 Effect of variation in leaf layer duration (ld) for area of *D. teres* (A) and *R. secalis* (B). ld values were varied between 10 (lowest line for both fungi) and 50 (uppermost line for both fungi) in intervals of 5 days. The horizontal line marks the value from the default epidemic parameter values (Table 4:4, Figure 4:3)

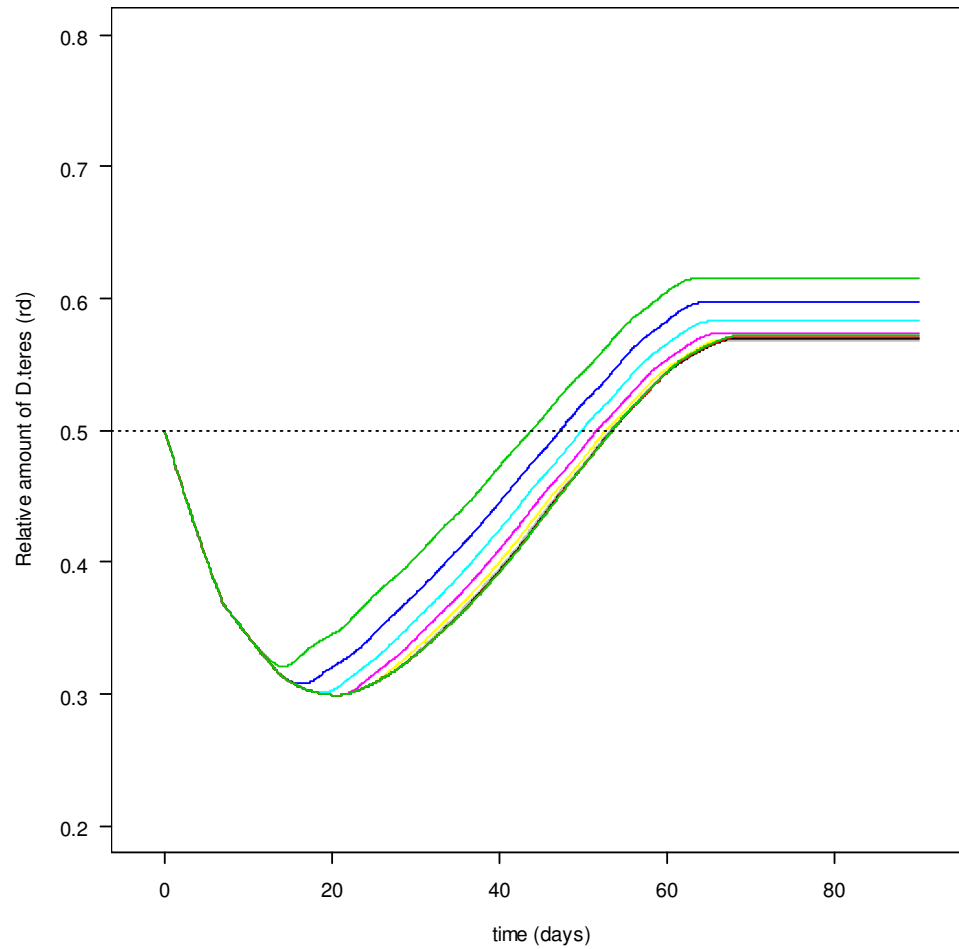


Figure 4:5 Sensitivity of the relative amount of D. teres of total disease to variation in the leaf layer duration (ld). ld values were varied between 10 (upper line) and 50 (lower line) in intervals of 5 days. The horizontal line marks $rd=0.5$: the value where diseased area is occupied by similar levels of the two pathogens.

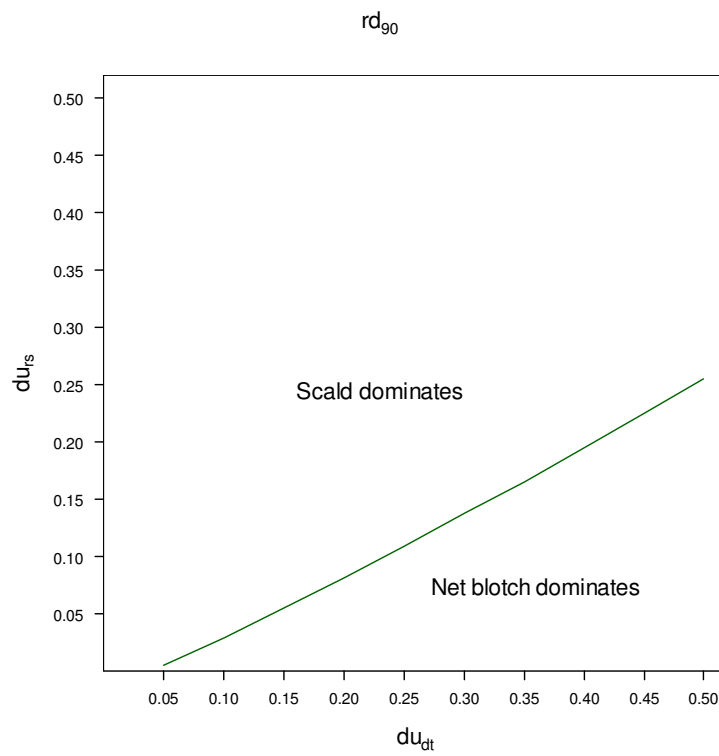


Figure 4:6 The relation between dispersal proportions of the two pathogens on rd after 90 days simulation. The line represents the combinations of the two dispersal parameters where rd_{90} is equal to 0.50. The area above the line are values where scald dominates, and net blotch below.

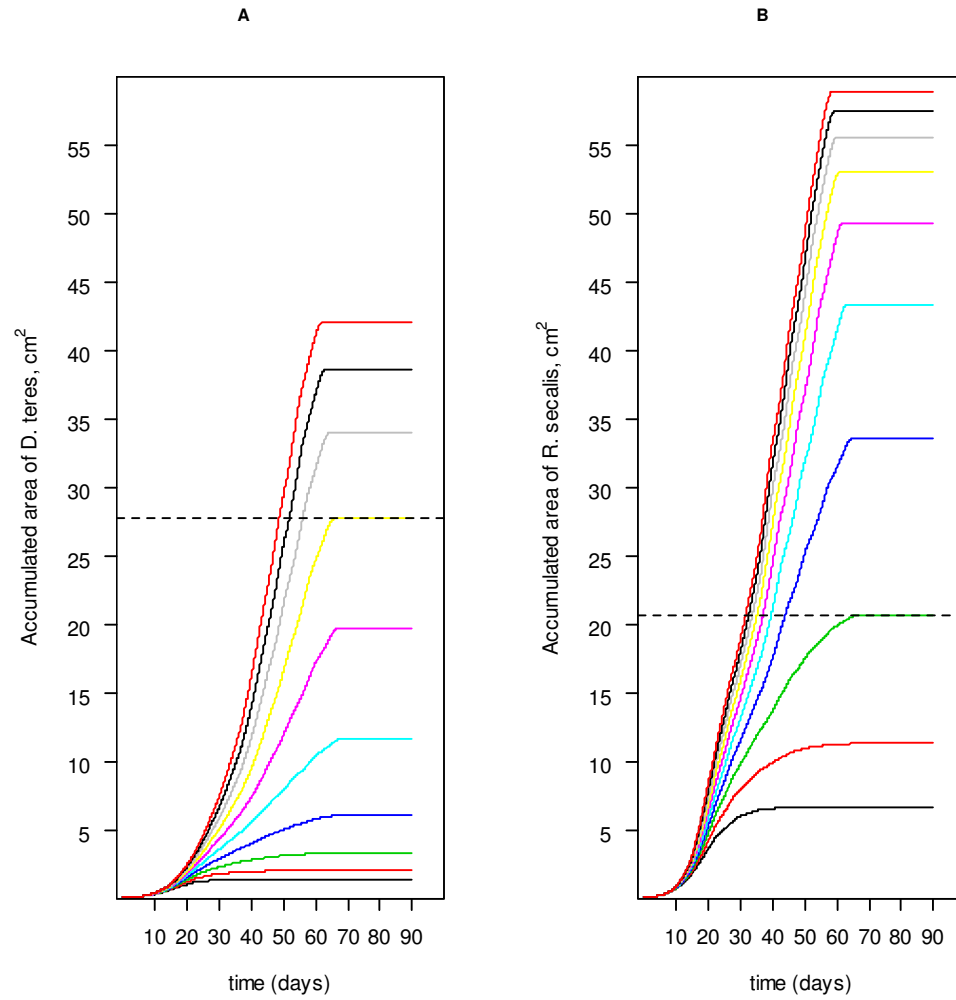


Figure 4:7 Developments in the levels of *D. teres* (A) and *R. secalis* (B) with different upward dispersal values (dd_i) ranging from 0 (lower lines) to 0.5 (upper lines) and the default value of the other species. The horizontal line marks the value from the default epidemic parameter values (Table 4:4, Figure 4:3)

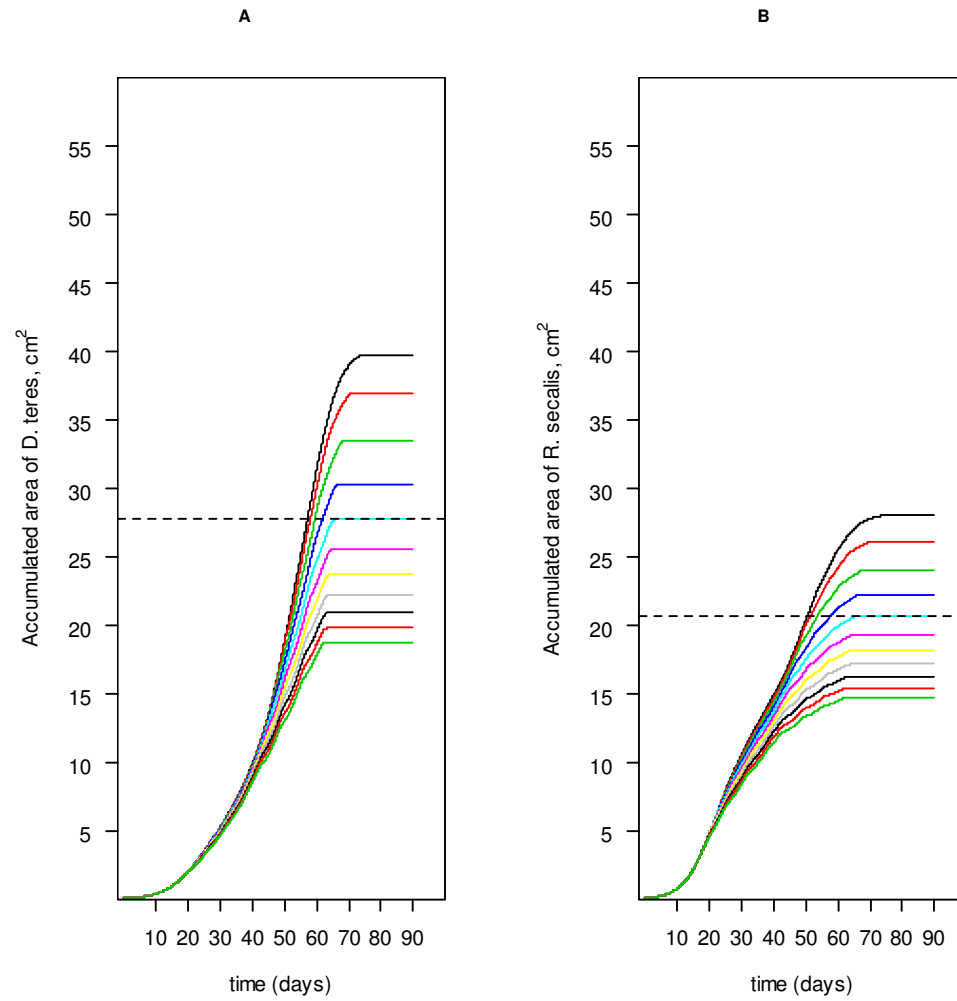


Figure 4:8 Developments in the levels of *D. teres* (A) and *R. secalis* (B) with different levels of disease effect on senescence (si) ranging from 0 (upper lines) to 0.5 (lower lines). The horizontal line marks the value from the default epidemic parameter values (Table 4:4, Figure 4:3)

5 Methods for assessing net blotch severity on spring barley and their relations to grain weight

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Extended Abstract

5.1 Abstract

Appropriate disease assessment methods and parameters reflecting whole-season disease severity levels in field plots remain important issues in studies related to plant disease epidemiology, disease resistance of crop cultivars, and disease-induced yield losses. Such methods and parameters should be yield-related to ensure relevance. Net blotch severity was determined over time in inoculated and non-inoculated field plots of three spring barley varieties by whole plot assessments and by assessments of individual leaves of single main tillers. Disease severity measures such as the area-under-disease-progress-curve, mean and maximum severity as well as severity levels at specific growth stages (GS) were derived from the data. Their relation to thousand grain weight (TGW) and their inter-correlations were examined by means of generalised linear model (GLM) and factor analyses (FA), respectively. All parameters of net blotch severity were significantly negatively correlated with TGW. Disease parameters derived from whole-plot assessments gave a slightly better explanation of TGW than parameters derived by assessing single main tillers. Net blotch severity at GS 70 (beginning of grain filling) of whole plot assessments yielded the highest adjusted R^2 (0.43) while the adjusted R^2 values resulting from using the same parameter of assessments of the upper three, four or all leaves of single tillers were between 0.34 and 0.35. Also, the residuals of TGW of GLM analyses using disease covariates from whole-plot assessments and variety effects as independent variables exhibited less pattern related to other sources of variation than residuals of the corresponding models that used single-tiller-based disease covariates. FA revealed that all disease parameters were highly inter-correlated and co-varied along the 1 principal component axis. The results indicate that disease assessments at GS 70 are appropriate to reflect whole-season severity levels of net blotch and that the time consuming single-tiller method is in this respect not superior to the simpler whole-plot method. However, assessing individual leaf layers of single tillers allowed to observe the epidemic development in great detail. This showed, for example, how much each leaf layer contributed at any given time to the total disease and revealed that a substantial fraction of the total disease is being removed during the course of an epidemic by

senescence of lower leaves. This level of detail in examining the dynamics of epidemics cannot be achieved by the whole-plot method.

5.2 Key words

Yield effects, yield loss, *Pyrenophora teres*, *Drechslera teres*, disease assessment methods, single-tiller assessment, single-leaf assessment, whole-plot assessment, disease parameters, intercorrelation, generalised linear model, factor analysis.

5.3 Materials & methods

The experimental details for the crop treatment, collection of plant samples and disease assessment on single leaves is described in Vollmer *et al.* (2005): chapter 3 this thesis.

From the treatments described in that paper, only data obtained from treatment 3 (Inoculation of *Drechslera teres* only in early May, the 'early' time point) and the variety Goldie are used in the present analysis.

5.3.1 Whole plot assessment

Disease assessment was made of each plot once every week during the trial (6 times during the season). Using a stick to separate the canopy, a visual assessment of amount disease in the crop was made. For each plot 3 recordings were made in different places. The observations were severity of all observed diseases separately (net blotch, scald, mildew, leaf rust) and percentage green leaf area of the canopy.

Ground cover was also observed. The same observer made all plot level assessments in both years. This observer was different to the observer who carried assessments on single leaves.

5.3.2 Yield parameter

Harvesting individual plots using a combine harvester was not possible, due to the size of the plots and the sampling for diseases assessments had left visible holes in the vegetation. For all plots 200 heads were therefore harvested by hand with a pair of scissors, heads were thrashed and kernels collected. Thousand grain weight (TGW) was calculated from the mass of 500 kernels.

5.3.3 Leaf area

To have an estimate of leaf area developments during the season, leaf measurements were taken from plants collected in the non-inoculated plots for the three varieties. Length (l_l) and width (l_w) were measured on the dried leaves on paper for all leaf layers. Leaves were selected that were disease free and green. The constant 0.776 is used to estimate the leaf area (Pons-Kühnemann, 1994) as:

$$\text{Leaf Area} = 0.776 \times l_l \times l_w$$

For the analyses it is necessary to determine a date for emergence of each leaf layer, which is estimated as the median value for the leaf development, i.e. the time point between no leaf and a fully emerged leaf layer.

5.4 Data preparation

The analysis included an estimate of disease levels on single tillers from the data on single leaves. For this all leaves for which data on leaf area existed were included in the analyses. The estimated percent net blotch severity values were converted into values representing cm^2 leaf area. The cm^2 healthy leaf area of individual leaves was computed

by subtracting the sum of diseased and necrotic leaf area from the total leaf area per leaf. The resulting absolute diseased-, necrotic- and healthy leaf area as well as the total leaf area of the individual leaves were then averaged across sub-samples (=plants) per leaf layer, plot and sampling date. Percent disease severities, necrosis and healthy leaf area per leaf layer, plot and sampling date across sub-samples were then computed by relating the absolute values of these variables to the total leaf area. The absolute total leaf area, healthy leaf area, diseased leaf area and necrotic leaf area per plant, plot and sampling date was computed by summing up the absolute values of the respective variables across leaf layers per plot and sampling date. The average percent healthy, diseased and necrotic leaf area per plant, plot and sampling date were computed by relating the absolute leaf areas to the absolute leaf area.

5.5 Results

Tables and figures from the analysis are presented on the following pages

5.6 Tables

Table 5:1 Adjusted R² values of relationships between observed 1000-grain weight and values estimated by GLM analyses to explain variation of 1000-grain weight based on: A) effects of covariates [x] representing various disease severity variables measured on different observation units in combination with variety effects [sources of variation: x, x · variety, variety]; B) other effects on residuals of A) [sources of variation: treatment, replication, variety · treatment, variety withing replication, treatment within replication]; C) both, A) and B).

covariate		adjusted R ²		
observation unit	disease severity variable	A) covariate & variety effects on total variation	B) other effects on residuals of A)	C) effects of A) + B) on total variation
whole plot	AUDPC	0.37	0.45	0.65
	mean	0.38	0.42	0.64
	maximum	0.38	0.35	0.60
	GS 70	0.43	0.44	0.68
	GS 50	0.28	0.59	0.71
	GS 30	0.17	0.62	0.68
whole main tiller	AUDPC	0.31	0.63	0.75
	mean	0.32	0.66	0.77
	maximum	0.33	0.68	0.78
	GS 70	0.34	0.66	0.78
	GS 50	0.22	0.65	0.73
	GS 30	0.19	0.67	0.73
upper 4 leaves of main tiller	AUDPC	0.32	0.63	0.75
	mean	0.37	0.68	0.80
	maximum	0.39	0.69	0.81
	GS 70	0.34	0.67	0.78
	GS 50	0.30	0.68	0.78
	GS 30	0.23	0.71	0.78
upper 3 leaves of main tiller	AUDPC	0.31	0.67	0.77
	mean	0.36	0.65	0.78
	maximum	0.34	0.61	0.74
	GS 70	0.35	0.66	0.78
	GS 50	0.25	0.68	0.76
	GS 30	0.17	0.67	0.73

Table 5:2 Effects tested by GLM analyses to explain variation of 1000-grain weight based on net blotch severity at growth stage 70 and variety. Left side: whole-plot assessments; right side: assessments of upper three leaves.

source of variation	assessed:					
	whole-plot			upper three leaves		
	mean square	df	sig. of F	mean square	df	sig. of F
corrected model	91.67	5	0.000	76.62	5	0.000
intercept	45371.46	1	0.000	78713.64	1	0.000
% net blotch severity	257.31	1	0.000	290.17	1	0.000
variety · % net blotch severity	74.89	2	0.000	102.14	2	0.000
variety	9.85	2	0.253	50.96	2	0.003
error	7.05	75		8.05	75	
total		81			81	
R ²	0.46			0.39		
adjusted R ²	0.43			0.35		

Table 5:3 Parameters estimated by GLM analyses to explain variation of 1000-grain weight based on effects of disease severity at growth stage 70 and variety. Left side: whole-plot assessments; right side: assessments of upper three leaves.

parameter	assessed:					
	whole-plot			upper three leaves		
	coefficient B	standard error of B	sig. of T	coefficient B	standard error of B	sig. of T
intercept	37.15	1.00	0.000	37.64	0.66	0.000
% net blotch severity	-0.46	0.33	0.175	-0.60	0.32	0.064
[variety=Brazil] · % net blotch severity	-0.11	0.45	0.810	0.24	0.51	0.646
[variety=Goldie] · % net blotch severity	-1.77	0.45	0.000	-3.07	0.66	0.000
[variety=Punto] · % net blotch severity	0 ^{a)}	.	.	0 ^{a)}	.	.
variety=Brazil	0.63	1.31	0.631	1.17	0.91	0.200
variety=Goldie	1.71	1.13	0.134	3.58	1.01	0.001
variety=Punto	0 ^{a)}	.	.	0 ^{a)}	.	.

^{a)} These parameters are redundant. Therefore, they are set to 0 and no statistics are computed.

Table 5:4 Effects tested by GLM analyses to explain variation of residuals of 1000-grain weight related to treatment, replication and variety. Left side: whole-plot assessments; right side: assessments of upper three leaves.

source of variation	assessed:					
	whole-plot			upper three leaves		
	mean square	df	sig. of F	mean square	df	sig. of F
corrected model	8.54	48	0.007	10.87	48	0.000
intercept	0.00	1	1.000	0.00	1	1.000
treatment	1.52	8	0.906	2.01	8	0.620
replication	121.56	2	0.000	137.64	2	0.000
variety · treatment	4.58	16	0.294	4.17	16	0.117
variety within replication	8.25	4	0.087	18.34	4	0.000
treatment within replication	3.04	16	0.654	5.64	16	0.028
error	3.70	32		2.56	32	
total		81			81	
R ²		0.78			0.86	
adjusted R ²		0.44			0.66	

Table 5:5 Results of factor analysis of disease severity variables measured on different observation units. Extraction method: principal component analysis.

		component		
		1	2	3
eigenvalue		19.3	1.3	1.2
% variance explained		80.4	5.6	4.9
cumulative % variance explained		80.4	86.0	90.9

observation unit	disease severity variable	communality extracted	component loadings		
whole plot	AUDPC	0.90	0.95	0.06	0.03
	mean	0.89	0.94	0.06	0.02
	maximum	0.76	0.87	0.03	-0.05
	GS 70	0.84	0.91	-0.04	-0.12
	GS 50	0.87	0.91	0.15	0.14
	GS 30	0.89	0.87	0.22	0.28
whole main tiller	AUDPC	0.98	0.96	-0.20	0.11
	mean	0.98	0.96	-0.22	0.06
	maximum	0.95	0.93	-0.31	-0.02
	GS 70	0.96	0.93	-0.31	0.01
	GS 50	0.91	0.92	-0.17	0.17
	GS 30	0.90	0.88	0.14	0.34
upper 3 leaves of main tiller	AUDPC	0.94	0.94	-0.23	0.02
	mean	0.97	0.88	0.22	-0.39
	maximum	0.96	0.85	0.18	-0.45
	GS 70	0.91	0.91	-0.30	-0.05
	GS 50	0.72	0.84	-0.05	0.08
	GS 30	0.83	0.72	0.46	0.31
upper 4 leaves of main tiller	AUDPC	0.98	0.96	-0.22	0.06
	mean	0.97	0.88	0.28	-0.35
	maximum	0.96	0.85	0.24	-0.43
	GS 70	0.96	0.93	-0.31	-0.03
	GS 50	0.88	0.92	0.16	-0.06
	GS 30	0.91	0.78	0.42	0.36

5.7 Figures

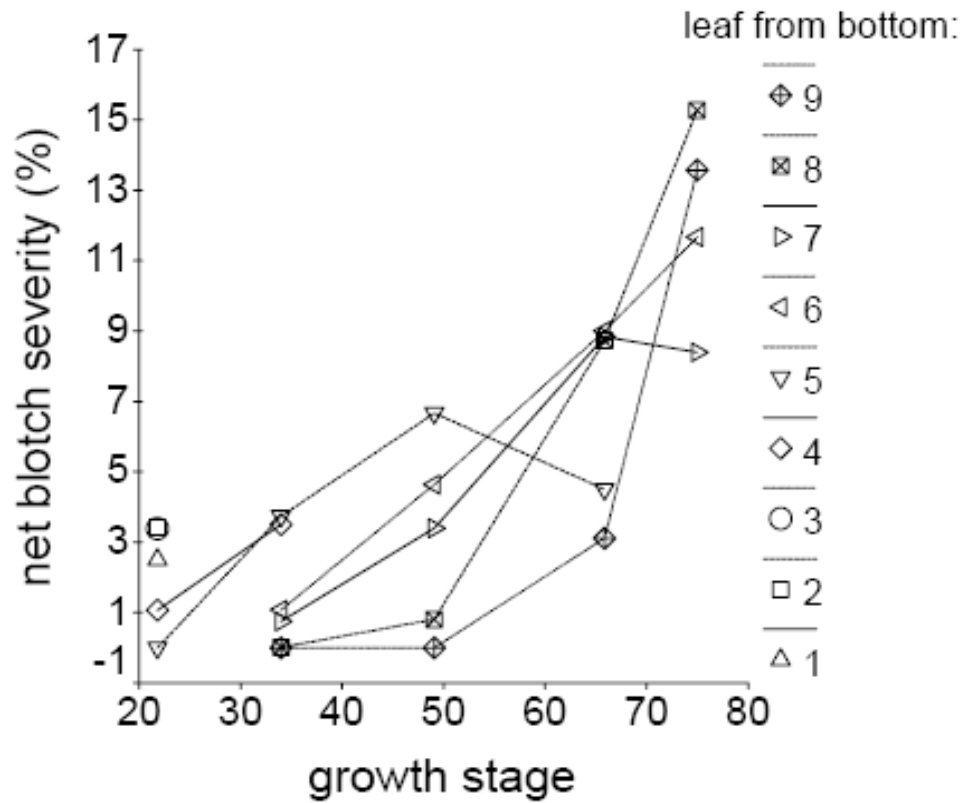


Figure 5:1 Progress of net blotch severity on individual leaves of the main tiller of variety Goldie. Leaves are numbered in order of their chronological appearance, from the bottom to the top of the tiller. Means of three replications of plots inoculated with net blotch infected straw at crop emergence.

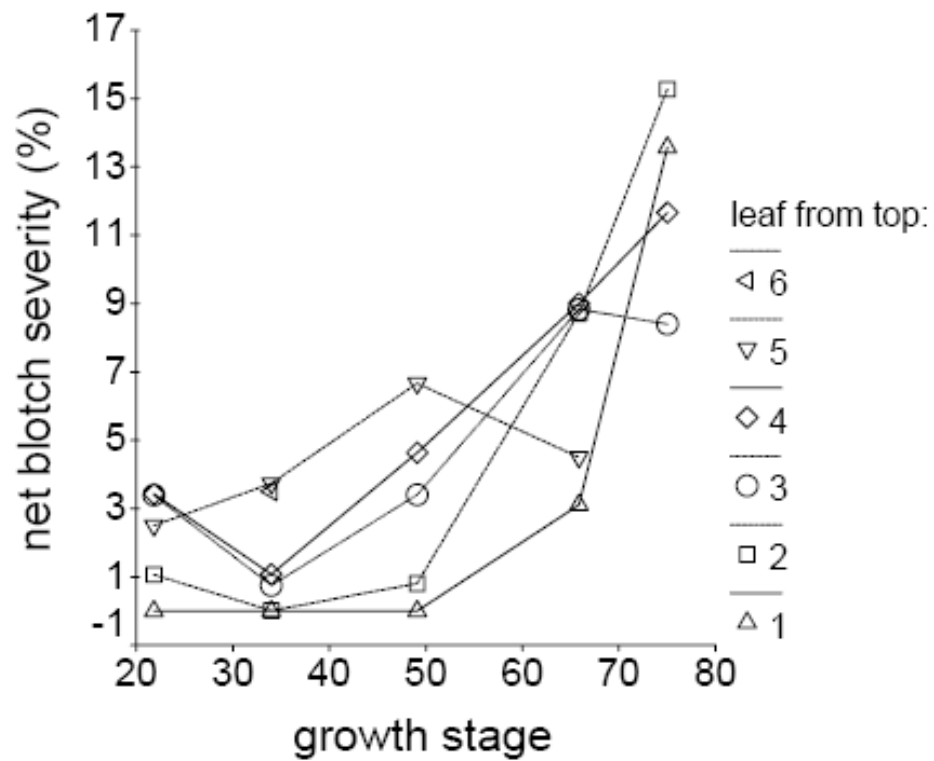


Figure 5:2 Progress of net blotch severity on individual leaves of the main tiller of variety Goldie. Leaves are numbered from the top to the bottom of the tiller. Means of three replications of plots inoculated with net blotch infected straw at crop emergence.

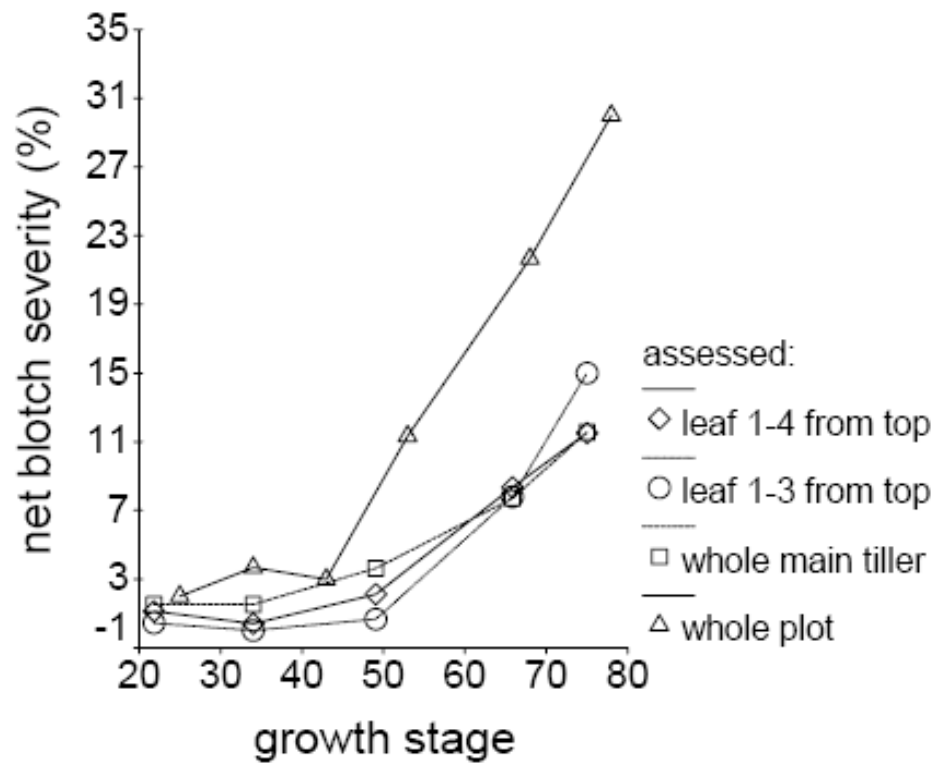


Figure 5:3 Progress of net blotch severity on variety Goldie as assessed on various observation units: whole plot, whole main tiller, leaf 1 – 3 of main tiller and leaf 1 – 4 of main tiller. Means of three replications of plots inoculated with net blotch infected straw at crop emergence.

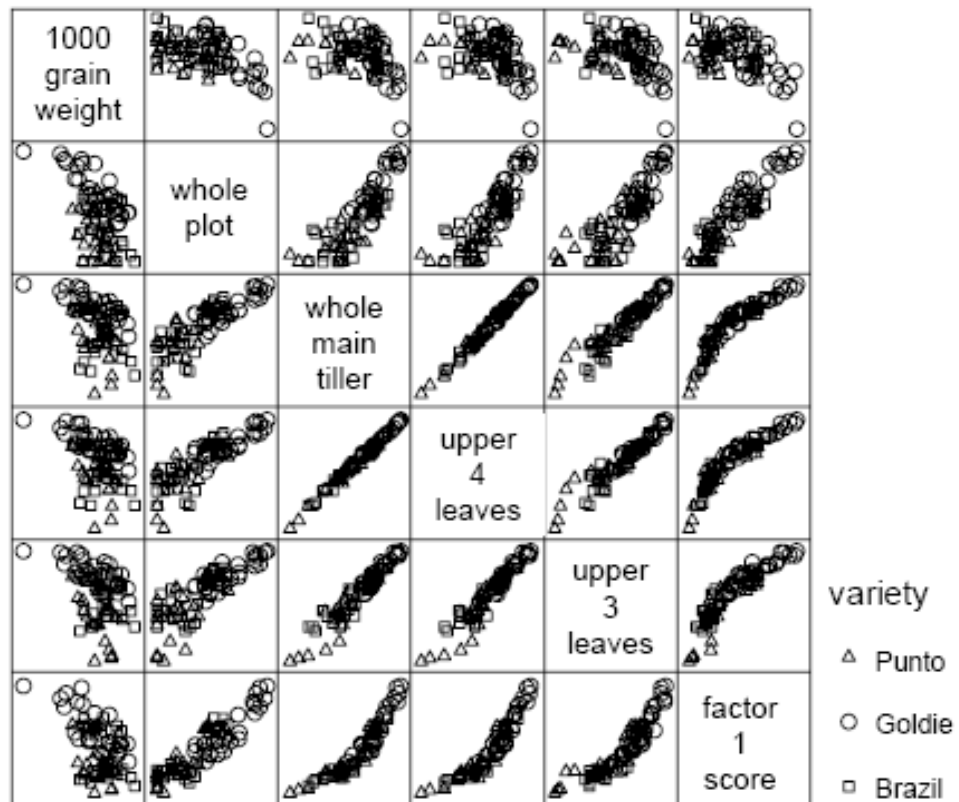


Figure 5:4 Thousand grain weight versus whole-plot assessments of net blotch severity at growth stage 70 and the corresponding net blotch assessments of the whole main tiller, leaf 1 – 3 and leaf 1 – 4 of the main tiller as well as scores of factor 1 of the factor analysis done on all disease parameters.

6 Outlook

The research presented in this thesis revolves around increasing the understanding of interactions between foliar fungal pathogens. The major contribution from the thesis is an increased understanding of how to approach this subject and analyse ensuing data. This information is highly relevant for all future research that will aim to explore multiple foliar diseases of any plant-pathosystem.

A major contribution is the review of studies of interactions between foliar pathogens. This is the paper that was lacking in the library when this project was initiated. To ensure research of the highest quality, it is vital to have a well-described theoretical background and hypotheses are formulated based on analysis of existing information. These are equally as important as the generation of new data. The field has seen a considerable amount of research, with a majority of studies starting off by emphasizing the importance of considering multiple pathogens, but these appear as single independent papers and as of yet no overall consideration of the subject has been published. The review has concluded that antagonistic and synergistic interactions are evident, but also that it is important with more research that has focus on natural field level dynamics.

The specific experimental study on *R. secalis* and *D. teres* confirmed previous studies on the simultaneous occurrence of the two pathogens, and showed the importance of considering disease development at the single leaf level of the plant. The paper showed that observing diseases on the single leaves provided information which is not available from crop level approaches, and thus that it is important to consider the crop dynamics together with the disease dynamics.

This aspect was further explored in the modelling paper. It was illustrated that observing interaction in the sense of synergism or antagonism between developing foliar pathogens, is a non-trivial task, if based on disease data from the field, as the competitive outcome from two simultaneously developing pathogens, even in the absence of antagonism or synergism, is difficult to predict due to influence from differences in dispersal parameters and crop development.

The two assessment methods: single leaf and the more generally employed whole-plot assessments were further explored and compared. The single leaf method observes epidemics in great detail in relation to the crop, but is also very resource consuming. The comparison gave valuable information on observations made by the plot method, which averages disease over the whole crop and summarizes processes related to dynamics of the crop as well as disease. However, this method did not provide, a better measure from which to explain yield, measured as thousand grain weight. Thus, this approach is perhaps mainly relevant in relation to exploring and understanding disease epidemiology, but for yield related purposes the full plot method is adequate.

From this it follows that the thesis has provided insight in some areas, through which many new research topics may be envisaged.

The conclusion from this thesis is that future research aiming to increase knowledge of interactions between foliar fungal pathogens must focus on disease development and dynamics in the field from natural inoculum. This is based on, among others, some of the conclusions from the review. One aspect is that interactions observed on inoculated plants in the greenhouse are generally less pronounced in the field, which may be related to the other conclusion, that timing of pathogen arrival is important. In the field it is less

likely that spores arrive in large concentrations in the manner of an inoculation experiment, based on which field inter-specific dynamics may not be adequately reflected in inoculation experiments.

Another aspect is that in most fields, diseases and pests probably count to more than two species. This forms a considerable challenge in terms of understanding the complete set of interactions, and improved understanding requires approaching it from several sides.

The specific interaction studies presented here, in the review and experimental paper, must be carried out along side more statistical approaches aiming to characterise the effect of the full range of pests which influence crop performance. The main information which is lacking today are data on how diseases develop naturally in the field, not from trials where pathogens have been applied, but disease data based on 'natural ' inoculum. Data must be taken on distribution of diseases on individual leaves on plants at different scales, from single plants to individual fields to geographical ranges covering many fields over different years. Preferably this is combined with spore trapping within and above the canopy, thus allowing correlation with future disease development. In addition attention should be paid to agricultural practise, including weeds, nutrient inputs, rotation scheme as well as plant susceptibility.

An approach like this requires collaboration between people from different disciplines. Analysis and interpretation requires collaborations between statisticians, agronomists and ecologists, to be able to pay suitable attention to dispersal ability, specific infection biology (leaves or grains), general pathogen biology and inter-season transmission ability. This type of data will provide information at the real population scale of the pathogens and thus natural associations, whether positive or negative, between pathogens. This thereby increases our knowledge on the importance of interaction between foliar pathogens, their relationship to varietal characteristics and thus provides guidelines for design of more specific experiments.

7 Appendix

7.1 Multiple diseases, host resistance and the role of variety mixtures for

Poster presented at 1. international symposium on organic seed production and plant breeding, Berlin, Germany, 21 - 22 Nov 2002; Published in *Proceedings of the 1. international symposium on organic seed production and plant breeding*, page 73.

Multiple diseases, host resistance and the role of variety mixtures for disease control in organically grown spring barley

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Background and objectives

One of the most important disease management components in organic farming is the use of disease resistant varieties. It is challenged by the presence of more than one disease that commonly occurs in farmers' fields at the same time. Because, while many modern spring barley cultivars possess good resistance to individual diseases, cultivars resistant to multiple diseases are still scarce since breeding for multiple disease resistance is a difficult and lengthy task. Using mixtures of cultivars possessing high levels of resistance to individual diseases may therefore be a viable option to control multiple disease complexes in organic farming. To ensure an efficient use of variety mixtures in managing multiple disease problems, the presence, nature and effects of interactions among the genotypes used in mixtures (Fig. 1) as well as among the occurring pathogens (Fig. 2) must be understood. The objectives of this work are to:

- 1) Evaluate the effects of variety mixtures, as compared to pure stands, in controlling the disease development of competing leaf pathogens.
- 2) Examine the interactions among competing leaf pathogens and their effects on the development of diseases complexes and crop performance.
- 3) Describe and model the dynamics of epidemics caused by competing leaf pathogens as affected by host resistance diversity.

Materials & methods

In a field trial with spring barley conducted on the organically cultivated area of the DIAS research farm in Flakkebjerg, Denmark in 2002, three variety treatments (1 - pure stand of variety Goldie, 2 - pure stand of variety Punto, 3 - Goldie and Punto in a 50%-50% mixture) were combined with four disease treatments (1 - non-inoculated control, 2 - net blotch inoculated, 3 - net blotch inoculated + scald inoculated at early tillering stage, 4 - net blotch inoculated + scald inoculated after tillering) in a fully randomised two-factorial design with three replications. The plot size was 2.5 x 2.5 m and the plots were surrounded by a 2.5 m border planted (to) with ?? oats. Net blotch inoculation was done by spreading infested winter barley straw after crop emergence. Wheat straw was spread on control plots. For scald inoculations, conidia suspensions were sprayed onto the plots. From tillering until flag leaf emergence, the plots were sprinkler-irrigated 3 x per day: 10 minutes in the evening, at night and in the morning, respectively, to provide optimum moisture conditions for sporulation, spore dispersal and infection. Crop growth stage, % diseased leaf area and % healthy leaf area were visually assessed in each plot 5 x during the growing season. The % net blotch- and scald severity as well as healthy leaf area averaged across observation dates were subjected to analyses of variance (ANOVA) and T-tests.

Results

Net blotch developed most rapidly in the plots inoculated with net blotch alone, followed by the plots inoculated with net blotch plus scald (Fig. 3, upper part). Substantial net blotch levels developed also in the non-inoculated control plots. Scald levels remained comparatively low in the whole trial. However, the late scald inoculation resulted in highest scald levels (Fig. 3, middle). The healthy leaf area was clearly higher in the non-inoculated control plots throughout the whole season, compared to the treatments inoculated with net blotch or net blotch plus scald (Fig. 3, lower part). The disease treatment factor thus had a dominant, highly significant effect on all independent variables (mean net blotch severity, mean scald severity and mean healthy leaf area). Interactions between the treatment factors were non-significant (Tab. 1). Parameterising the disease treatment effects indicated that the inoculation with net blotch alone clearly resulted in highest mean net blotch severity and lowest mean healthy leaf area, followed by the treatments that included scald inoculation (Tab. 2, lower part). The late scald inoculation resulted in the highest mean scald severity levels. Although the variety treatment factor had a significant effect only on the mean net blotch severity (Tab. 1), the parameterisation of the variety treatments (Tab. 2, upper part) indicated a lower mean net blotch severity and a higher mean healthy leaf area of the variety mixture, as compared to the average of the pure stand treatments.

Conclusions and outlook

The first years' results showed that occurrence of both diseases in combination led to less-than-additive effects on disease severity of the predominant disease (net blotch) and crop performance (healthy leaf area) as compared to single-disease scenarios. This indicates antagonism between the disease organisms and has direct implications for disease-yield loss relationships and yield loss appraisal. There were hints for higher-than-additive effects of variety mixtures in reducing disease severity and improving overall crop performance. This indicates potential benefits of using variety mixtures, as compared to pure stands, in controlling disease complexes. The study will be expanded to include additional varieties and their mixtures as well as more aspects regarding the time of establishment and amount of initial inoculum of the two pathogens. The data will be used to develop a simulation model for the development of the two competing diseases as affected by resistance properties of individual varieties and variety mixtures and to derive decision aids for optimising the use of variety mixtures in the management of multiple diseases.

Figure 1. Growing varieties in mixture: hypothetical effects on crop performance. If varieties grown in mixture do not interact, the relationship between mixing ratio and crop performance will be linear. Antagonism among varieties leads to less-than-additive-, synergism to greater-than-additive effects.

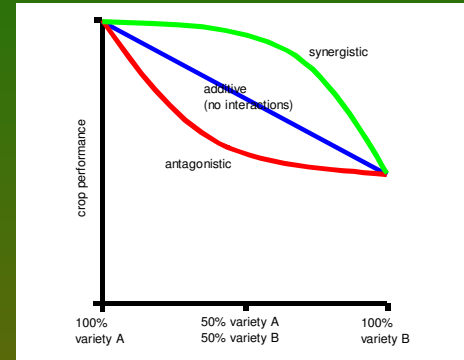


Figure 2. Examples of types of competition between two pathogens, A and B. The green circle symbolises the metabolic pool of the crop that is available to the pathogens. The blue and red ovals stand for the portions of the metabolic pool that will be allocated to the respective pathogen.

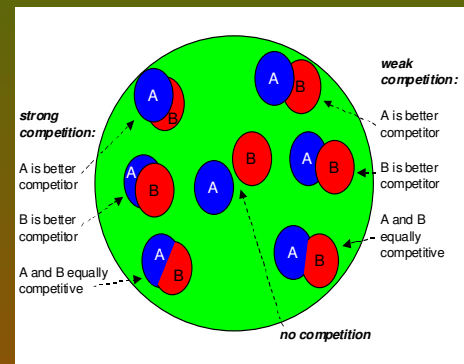
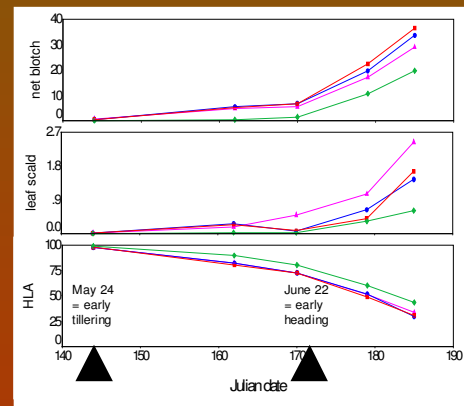


Figure 3. Development of % net blotch severity, % leaf scald severity and % healthy leaf area by disease treatment across variety treatments.



Disease treatment:
 - control (non-inoculated)
 - net blotch inoculated
 - net blotch + early scald inoculated
 - net blotch + late scald inoculated

Table 1. ANOVA results: significance levels (p) of F values of variety- and disease treatments and replication with respect to net blotch- and scald severity and healthy leaf area averaged across observation dates.

source of variation	dependent variable:		
	net blotch severity	leaf scald severity	healthy leaf area
variety treatment (V)	0.025	0.613	0.057
disease treatment (D)	0.000	0.015	0.000
replication	0.393	0.002	0.018
V * D	0.145	0.154	0.411

Table 2. ANOVA results: treatment parameters and their significance levels (T-test) as compared to the reference treatment; ns = non-significant; * = significant at p = 0.05 and 0.01, respectively.

treatment	dependent variable:		
	net blotch severity	leaf scald severity	healthy leaf area
variety:			
Goldie	-1.579 ns	-0.538 ns	-0.889 ns
Punto	3.066 ns	0.202 ns	-2.000 ns
mixture (= reference)	0.000	0.000	0.000
disease:			
control (non-inoculated)	-2.587 ns	-0.695 *	3.600 ns
net blotch	5.644 **	-0.618 ns	-5.044 *
net blotch + early scald	2.100 ns	-0.514 ns	-2.733 ns
n. b. + late scald (= ref.)	0.000	0.000	0.000

7.2 Studies of interaction between pathogens

Workshop presentation at the 11th International Cereal Rusts and Powdery Mildews Conference, John Innes Centre, Norwich, England: 22nd to 27th August 2004

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Abstract

A fundamental ecological observation is that activity of any organism affects the environment in which it lives. This has consequences not only for that organism or members of that species, but for other species inhabiting the same environment. It means that the dynamics of a population of a species living in a given environment cannot be evaluated without considering other species in the same environment and the interactions with and between these. Two species may experience several potential interaction types, e.g. competition (A and B have a negative effect on each other), mutualism (A and B have a positive effect on each other) or exploitation (A has positive effect on B, while B has negative effect on A), which may be either direct or indirect.

Pathogen interactions and their effects on disease levels are often ignored in plant pathology, where pathogens are most often studied in isolation. Here we will discuss how to infer interaction from data. We will present a list of studies that consider two diseases simultaneously and discuss the use of different approaches of analysis to demonstrate interaction. Among these approaches is the use of the Lotka-Volterra model for competition between two species, the implications of which will be considered. We will discuss the difference between interaction and density dependence.

7.3 Simultaneous epidemic development of scald and net blotch on single leaf layers of a spring barley crop

Poster presented at 9th international workshop on plant disease epidemiology,
Landerneau, France, 11-15 April 2005

Simultaneous Epidemic Development of Scald and Net Blotch on Single Leaf Layers of a Spring Barley Crop

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Background & Objectives

Two pathogens growing on the same leaf compete for the same resources, i.e. space and plant nutrients, which may lead to different types of interactions. The importance of such interactions for epidemics of simultaneously occurring pathogens has received little attention.

The objective of this study is to investigate the epidemics of scald (caused by *Rhynchosporium secalis*) and net blotch (caused by *Drechslera teres*) when occurring together on spring barley leaves in the field.

Here, data are presented focusing on the predominant disease net blotch.

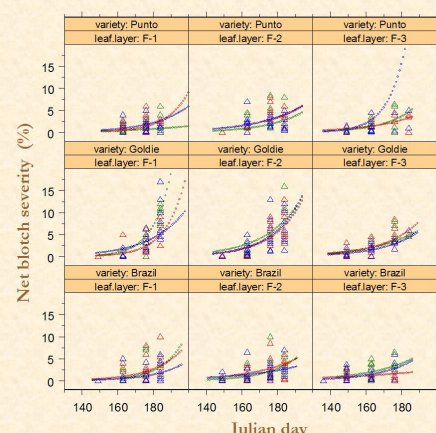


Fig 1. Disease severity over time on individual leaves. 3 varieties, 3 leaf layers and 3 treatments, for colour code see Materials & Methods. Lines show exponential model fit. Data from one replicate.

Material & Methods

Field trial

- D. teres* and *R. secalis* were applied artificially in different combinations and timing (6 treatments) in field plots of 3 spring barley varieties in 3 replications. Here only 3 treatments are considered:
- 1: *D. teres* at Julian day 123 (2-3 leaf stage)
- 2: *D. teres* + *R. secalis* at Julian day 123 (2-3 leaf stage)
- 3: *D. teres* at Julian day 123 (2-3 leaf stage) + *R. secalis* at Julian day 149 (6-7 leaf stage)

Data collection

- 9 plants were harvested from each plot 5 times during the season
- Leaves were dried. Disease severity and senescence observed.
- Only leaves with < 50 % senescence are included in the analysis.
- Only results for leaf layers F-1, F-2 and F-3 are shown (F = flag leaf).

Analysis

- Different models were fitted to severity data over time for each leaf layer per variety, treatment and replication. An exponential model ($y(t) = y_0 \cdot \exp(x \cdot t)$) gave the best fit. Only the growth rates (x) are considered here.
- Association between scald and net blotch severity on individual leaves from the last 3 observation dates was estimated by Kendall's τ per leaf layer and variety. Only leaves from plots inoculated with both pathogens at Julian day 123 (Treatment 2) were included.

Results

Fig. 1: Net blotch developed on all leaf layers but scald developed very little on upper leaf layers (data not shown). On individual leaves, disease severity levels up to 30 % for net blotch (data shown for one replicate only) and 10 % for scald were observed (data not shown).

Fig. 2: Growth rates of net blotch per leaf layer were significantly affected by variety and, for variety Goldie, by leaf layer. There was a slight tendency for growth rates to be lower in the presence of scald in the crop (Treatment 2 + Treatment 3) (test not shown). Treatments had a significant effect on initial disease severity in the complete field trial (data not shown).

Fig. 3: Significant negative associations between the severity of the two diseases on individual leaves for several combinations of leaf layer and variety were observed.



Scald
Net blotch

Discussion & Conclusion

- The disease development curves on individual leaves were best described by an **exponential model**.
- Reduced growth rates** were implicated for net blotch in the presence of scald in the crop although this effect was not significant.
- Significant negative associations** between the two diseases on individual leaves were revealed in the association analysis, confirming indications above.
- This suggests an **interaction** between the two pathogens.
- The **mechanism** behind this interaction could be induced resistance in the plant.
- These results show that the **individual leaf approach** can provide new information and highlights the importance of considering interactions between pathogens in the field.

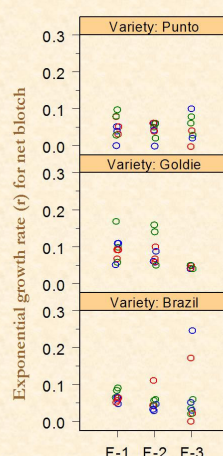


Fig. 2 Exponential growth rates categorised by treatment, leaf layer and variety. Colour code in Materials & Methods.

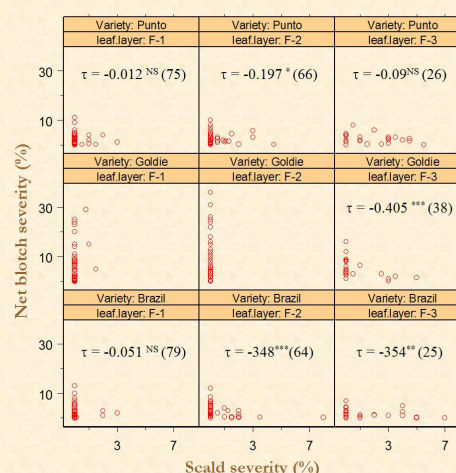


Fig. 3 Association between net blotch and scald severities on individual leaves. Kendall's τ (sample size). Data from Treatment 2 for the last 3 sampling dates

Mission

To promote an innovative and environmentally sustainable technological development within the areas of energy, industrial technology and bioproduction through research, innovation and advisory services.

Vision

Risø's research **shall extend the boundaries** for the understanding of nature's processes and interactions right down to the molecular nanoscale.

The results obtained shall **set new trends** for the development of sustainable technologies within the fields of energy, industrial technology and biotechnology.

The efforts made **shall benefit** Danish society and lead to the development of new multi-billion industries.